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"Council on Pharmacy & Chemistry
Therapeutic Research Committee"

ANNUAL REPORT (REPRINTED PAPERS) OF THE INVESTIGATIONS CARRIED OUT UNDER THE SUPERVISION

OF THE

THERAPEUTIC RESEARCH COMMITTEE

OF THE

COUNCIL ON PHARMACY AND CHEMISTRY

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RESEARCH INVESTIGATIONS

THE RATE OF ABSORPTION AND EXCRETION OF THE IODIDS OF STRONTIUM, SODIUM AND POTASSIUM *

E. J. KRAHULIK, M.D.
OMAHA

AND

J. D. PILCHER, M.D.
CLEVELAND

(From Archives of Internal Medicine, January, 1918.)

Strontium salts have been used to a limited extent in therapeutics for the effect of the bromid, iodid or salicylate radical, but their use has been more or less discouraged because it was thought that the strontium salts were more slowly absorbed than the corresponding salts of sodium and potassium.[1]

Since all iodids would exist in the blood and tissues essentially as sodium iodid, the excretion would be the same irrespective of the cathion, provided, of course, that the other conditions were the same. Consequently, any difference in the excretion of the iodid in the urine would indicate that the change was primarily in the absorption of the original iodid. Iodid was chosen in this investigation because its normal excretion has often been determined and because its quantitative estimation is simple and accurate. The excretion of strontium iodid was compared with that of sodium and potassium.

Method.—The investigation was made chiefly on one subject, but for comparison a lesser number of experiments were made with the strontium and sodium salts, only, on two other individuals. Each subject led a fairly uniform life during the investigation. The iodid was taken immediately after a uniform breakfast and the total urine collected at the following periods: ½, 1, 2, 6, 12, 24, 48 and 72 hours. At least three experiments were made with each drug, with a minimum interval of four days between experiments to insure the complete excretion of the previous dose. The iodid was estimated by the usual methods.[2] The averages of the iodid excreted at the different periods and the total excretion are given in Table 1; the percentages in Table 2. The results are graphically illustrated in Figures 1 and 2. (As the results were similar in the different subjects the excretion of one, only, is given in the figures.)

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* From the Pharmacological Laboratory, School of Medicine, University of Nebraska.

* The investigation was undertaken at the request of the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

[1] Cushny: Pharmacology and Therapeutics, 1915, p. 567.

The iodid content of the salts as determined by analysis was as follows:

NaI — 0.784 gm. per gm.
SrI — 0.525 gm. per gm.
KI — 0.722 gm. per gm.

TABLE 1.—QUANTITY OF IODIN IN THE URINE AT THE SEPARATE PERIODS, AND TOTAL EXCRETION IN GRAMS OF IODIN

Hour	NaI 1 Gm.	Total	SrI 1 Gm. (x Factor)	Total	SrI 1.5 Gm.	Total	KI 1.09 Gm.	Total	NaI* 1 Gm.	Total	SrI* 1 Gm. (x Factor)	Total
½	0.0063		0.0057		0.0053		0.0082					
1	......		......		......		......		0.0298	0,0298	0.0291	0.0291
2	0.0871	0.0934	0.0816	0.0873	0.1103	0.1156	0.1022	0.1104	0.0581	0.0879	0.0897	0.1188
6	0.2023	0.2957	0.2116	0.2989	0.2181	0.3336	0.2197	0.3301	0.2182	0.3061	0.2175	0.3363
12	0.1562	0.4519	0.1748	0.4737	0.1791	0.5126	0.1705	0.5006	0.1700	0.4765	0.1400	0.4763
24	0.1524	0.6043	0.1268	0.6005	0.1731	0.6858	0.1545	0.6551	0.1288	0.6053	0.1753	0.6516
48	0.0771	0.6813	0.0942	0.6948	0.0861	0.7719	0.0796	0.7347	0.0542	0.6595	0.0540	0.7056
72	0.0108	0.6921	0.0130	0.7078	0.0162	0.7881	0.0239	0.7586				

* Subject P.; all others from Subject K.

---

[2] Convenient quantities of urine (10 to 50 c.c.) were evaporated in nickel crucibles with 1 gm. potassium hydroxid and 0.5 gm. potassium nitrate per 10 c.c. of urine; fused and dissolved in water; made acid with 10 per cent. sulphuric acid and 10 drops of 10 per cent. sodium nitrite added, and the liberated iodin extracted with carbon disulphid and titrated with hundredth normal thiosulphate.

TABLE 2.—AVERAGE TOTAL IODIN EXCRETION IN PERCENTAGE OF THE INTAKE IN GRAMS OF IODIN

Hour	NaI 1 Gm.	SrI 1 Gm.	SrI 1.5 Gm.	KI 1 Gm.	NaI[*] 1 Gm.	SrI[*] 1 Gm.
½	0.8	0.72	0.68	1.0	....	....
1	....	....	....	....	3.8	3.7
2	11.8	11.1	14.7	14.1	11.2	15.2
6	37.7	38.0	42.5	42.1	39.0	42.6
12	57.6	60.1	65.4	63.8	60.8	60.8
24	72.8	76.5	87.5	83.5	77.2	82.8
48	82.6	88.4	98.4	93.7	84.1	90.0
72	88.3	90.3	100+	96.7		

[*] Subject P., all others from Subject K.

One gram of sodium iodid was the standard dose; 1.5 gm. of strontium iodid and 1.09 gm. of potassium iodid gave equal amounts of iodin. In several experiments but 1 gm. of strontium iodid was taken, so that in the tables and figures the results are multiplied by the correct factor as indicated.

Rate of Excretion.—There is practically no difference in the rate of excretion of the iodids of strontium, sodium and potassium as illustraed in Figure 1, which shows an increasing excretion rate reaching the maximum at about the sixth hour and then decreasing rapidly; during the third day but 10 to 20 mg. were excreted in all.

Total Excretion.—A somewhat greater quantity of the strontium iodid than of the sodium iodid was excreted at the twenty-four, forty-eight and seventy-two hour periods; the potassium iodid (one subject) excretion lay between the strontium series. As can be seen from the tables and figures, the difference is slight and probably of no significance.

There seemed to be no definite ratio between the excretion of the iodid and the volume of urine. There were instances in which a greater volume of urine was associated with a greater excretion of iodid, but the reverse was about as frequent. The point is well illustrated in one instance of the potassium series in which the iodin excretion was strikingly uniform at the sixth hour period (225, 212 and 222 mg.) with a marked variation in the urine output (600, 205 and 270 c.c., respectively). The average volume of urine was practically the same with the different salts.

Subjective Symptoms.—About an hour after taking the iodid two subjects noticed an irritation or soreness of the pharynx which persisted for several hours and disappeared during the afternoon. There was no apparent difference from the different iodids in the time of onset, intensity or duration of the symptoms. No other symptoms were noted. The third subject noted an acne eruption of the same degree from the strontium and sodium salts.

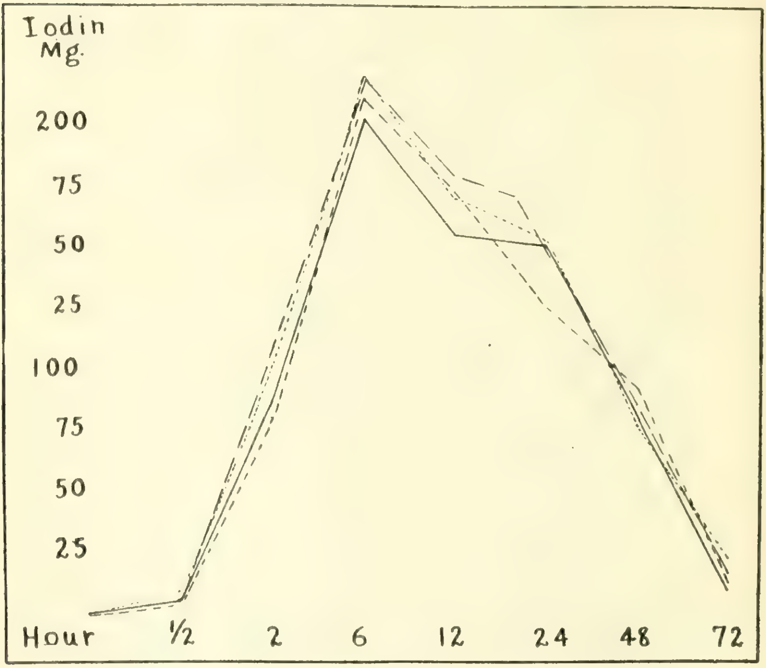

Fig. 1.—Rate of excretion of iodin in milligrams (Subject K); ——— sodium iodid; .... potassium iodid; — — — — strontium iodid; - - - - - - strontium iodid x factor.

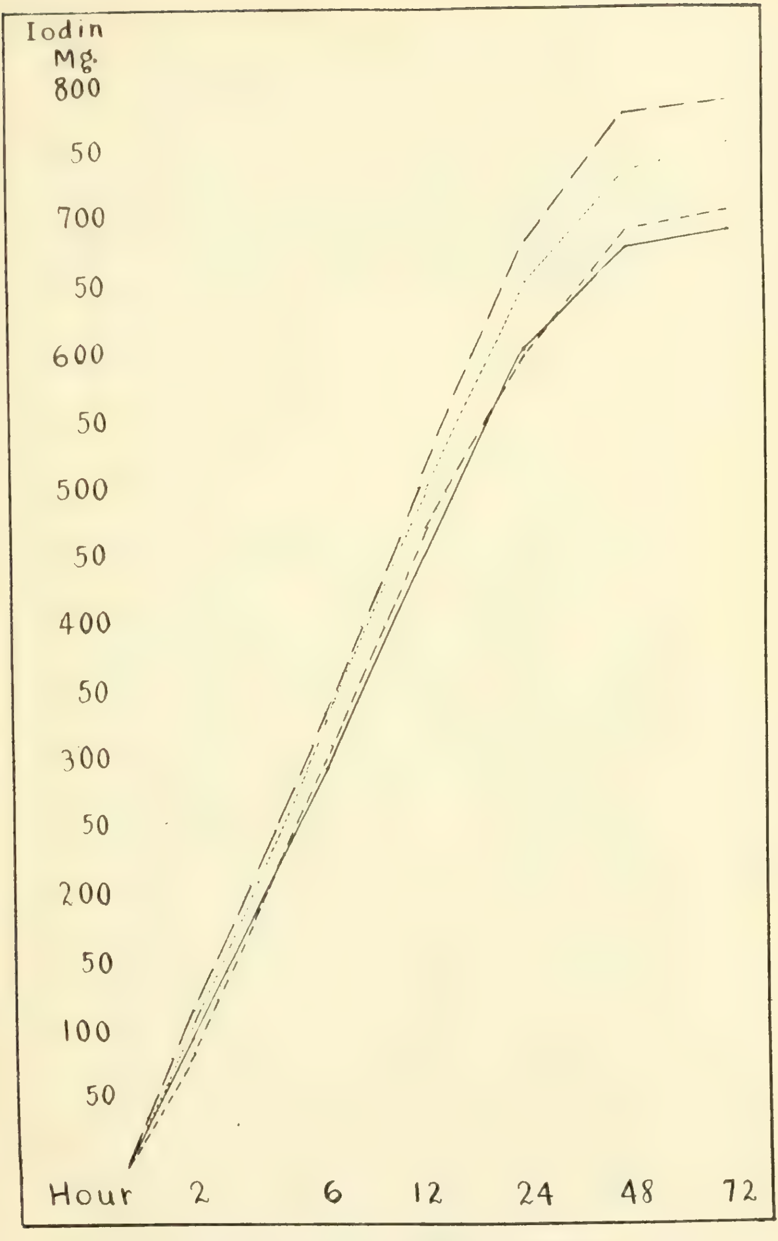

Fig. 2.—Total excretion of iodin in milligrams (Subject K); ——— sodium iodid; .... potassium iodid; — — — — strontium iodid; - - - - - - strontium iodid x factor.

CONCLUSIONS

The combination of iodin with strontium does not delay the excretion, and therefore the absorption, of iodin over that of sodium and potassium. In fact, both the rate and total excretion seem to be slightly greater with the strontium iodid, although the difference seems immaterial.

THE COMPARATIVE EFFICIENCY OF LOCAL ANESTHETICS *

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CLEVELAND

(*From The Journal A. M. A., Jan. 26, 1918, p. 216*)

Part I. The Investigation of the Clinical Efficiency of Local Anesthetics by Experimental Methods

Criteria of Usefulness.—The substances that have been introduced as local anesthetics are so numerous that it is difficult properly to compare their value. This has been attempted in a considerable number of experimental investigations; but the results have rather confused than cleared the subject. The confusion is due largely to faulty interpretation and unwarranted extension of the results into fields to which they do not apply, or to the failure properly to analyze and reproduce the essential factors of the clinical use of these agents.

The clinical desirability of a local anesthetic is determined on the one hand by its anesthetic efficiency, and on the other hand by the degree of local irritation and by the systemic toxicity.

Systemic toxicity has usually been estimated by hypodermic injection into guinea-pigs. The value of this method is extremely doubtful. The choice both of the animal and of the channel of administration is unfortunate from a clinical standpoint. Rodents depart much more widely than do dogs or cats from human subjects in their response to various poisons. They are, therefore, of questionable value even for the determination of *absolute* toxicity. *Actual* toxicity further involves the rate of absorption. Rapid destruction renders this especially important for the local anesthetics. Most cases of human poisoning arise from application to mucous membranes. The absorption from these, of course, has no relation to the absorption from hypodermic injections in guinea-pigs.

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* From the Pharmacological Laboratory of the Western Reserve University School of Medicine.

* This investigation was partly supported by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

Local irritation is best estimated by clinical application. In fact, clinical experience soon limited the use of the more irritant of the anesthetics, such as stovain, acoin, and the higher concentrations of quinin-urea hydrochlorid.

It seemed advisable to divide the problem, so that the present investigation does not touch further on the questions of irritation and toxicity, but is confined to the subject of anesthetic efficiency.

The methods most widely used for the estimation of anesthetic efficiency consist in observing: (1) the abolition of motor irritability of the frog's sciatic nerve, immersed in the solutions; (2) the failure of the frog, intact or, better, decapitated, to retract the irritated foot, after its immersion in the solution; (3) abolition of the winking reflex after application to the cornea, and (4) abolition of sensation after intracutaneous injection in the human subject.

Many series of determinations have been made with one or several of these methods, the efficiency being judged sometimes by the minimal efficient concentration, and sometimes by the duration of the effect, but often without a clear understanding of the relation of these various experimental methods to the various clinical methods.

METHODS OF ESTIMATING ANESTHETIC EFFICIENCY

Local anesthetics are used clinically in several very distinct ways, especially on mucous surfaces (surface anesthesia); by infiltration and hypodermic injection (injection anesthesia); by intraneural injection, and by subdural (spinal) injection.

Each of these clinical methods involves not only the factor of the *absolute* efficiency but also the balance between the penetration to the nerve fibers, and of absorptive removal from the anesthetic region.

With these considerations in mind, I have employed and compared all these experimental methods, and a further method of direct application to the sensory fibers of the sciatic trunk (testing their excitability by the persistence of the reflexes). The results appear to justify definite conclusions as to the scope of these methods, and as to the availability of the tested anesthetics for clinical purposes.

1. The application of the anesthetics to the motor fibers gives concordant results that are of scientific interest; but these results cannot be transferred to sensory fibers and therefore have little practical value.

2. The sensory methods fall into two classes: (*a*) the application to mucous surfaces, illustrated by the cornea or frog-skin and corresponding to the clinical use in the eye, nose, throat, urethra and bladder, and (*b*) the direct application of the anesthetic to the nerves, either by injection, or by immersion of the exposed nerves. The methods of the second class (*b*) are illustrated, respectively, by the intracutaneous (wheal) method, and by the application to the sensory nerve of the frog.

SCOPE OF THE VARIOUS EXPERIMENTAL METHODS

Experimental Method	Adaptation to Clinical Problems
Intracutaneous	Absolute anesthetic efficiency Infiltration and injection anesthesia Intraneural injection
Intracutaneous and sensory nerve trunk	Suggestive for subdural injection
Cornea and frog-skin.	Anesthesia of mucous membranes (the frog-skin test being less satisfactory because of the absence of circulation, and the different structure of the mammalian mucosae).

The intracutaneous method gives the closest approximation to the absolute anesthetic efficiency: it does away entirely with the factor of penetration, since the solution comes into direct contact with the fine nerve-fibers. Absorptive removal of the drugs can be practically obviated, either by making the observations very shortly after injection, or by the addition of epinephrin.

In addition to this scientific value, the wheal method is important because its results can be transferred directly and quantitatively to the clinical method of infiltration and intraneural anesthesia.

Immersion of the nerve trunk into the anesthetic solution, and testing the response of the sensory fibers, seem to approach closely the clinical methods of subdural injection. However, the clinical conditions are complicated by the undetermined factor of the rate of penetration in human spinal nerves; by the absorptive removal, and by the reaction of the cerebrospinal fluid. It is conceivable, therefore, that the order of clinical efficiency for intraneural injection may differ more or less from the experimental efficiency, by either the intracutaneous or the nerve-trunk method. These, therefore, can be considered only as suggestive.

In the light of the foregoing, the scope of the various experimental methods may be defined as in the accompanying tabulation.

PART II. SYNOPSIS OF THE RESULTS OF THE INVESTIGATIONS

The methods of experimentation and analysis, as sketched in Part I, have been applied to those local anesthetics that appear to have maintained themselves in clinical practice, and also to a few other substances which have not been used extensively in the clinic, but which appear worth investigating from the same standpoint.

I shall present the results mainly in the form of diagrams, dispensing with the details. These can be found in the full papers published in the *Journal of Pharmacology and Experimental Therapeutics.*

EFFICIENCY RATIO OF THE ANESTHETICS

This is shown in Figures 1 and 2. Figure 1 represents the methods that reproduce injection anesthesias.

Figure 1*A* shows the results of the intracutaneous method, indicating the absolute efficiency; it corresponds also to the practical efficiency for infiltration, injection and intraneural anesthesia.

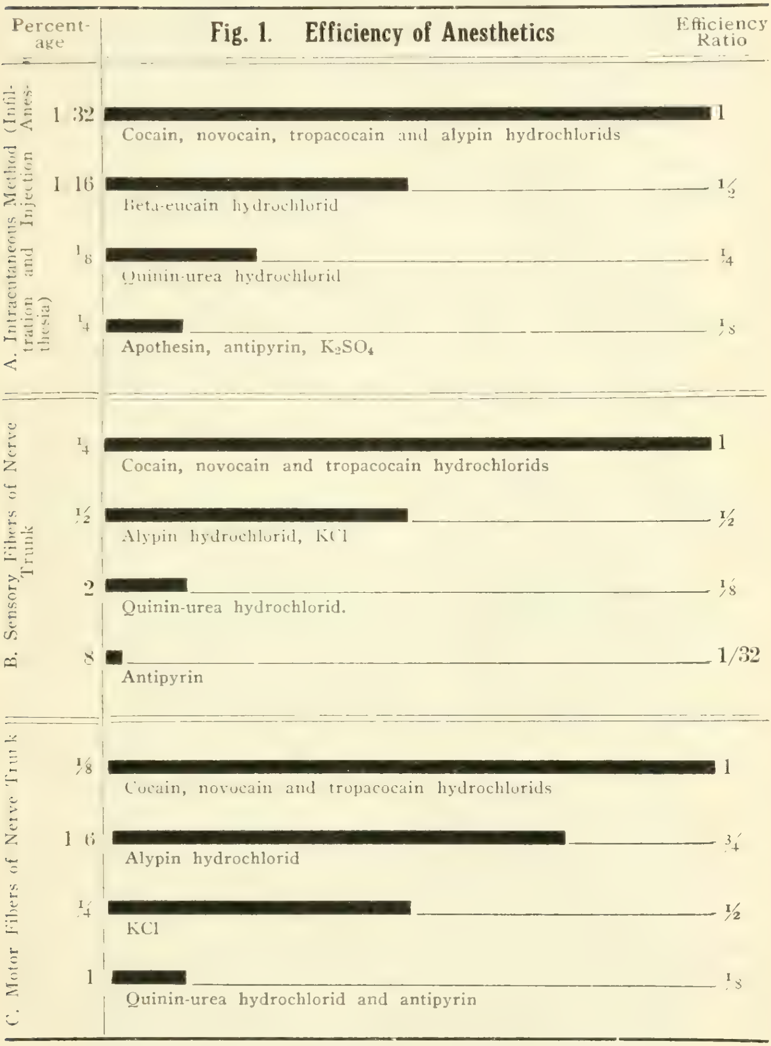

Fig. 1.—Efficiency of anesthetics in injection anesthesias.

Figure 1*B* shows the results of the direct immersion of the nerve trunk, for the sensory fibers. It probably approaches the conditions of subdural injections (potassium chlorid may behave differently on subdural injection).

Figure 1*C* gives the corresponding results for the motor fibers. They agree fairly well with the sensory fibers.

Figure 2 represents the methods of surface anesthesia.

Figure 2*A* shows the results for the cornea, which is probably fairly representative of mucous membranes.

Figure 2*B* gives the results with the frog-skin, which are in the same class, but are less related to the clinical conditions.

Rapidity of the Onset of Action.—This has no practical importance for infiltration anesthesia, since the action is almost instantaneous with this method. The rapidity is somewhat more significant for surface anesthesia, but even here the differences do not appear to have much practical importance. For the cornea, with the minimal effective concentrations, the anesthetics (hydrochlorids) could be arranged in three groups:

Most rapid: Tropacocain, alypin, quinin-urea.
Intermediate: Beta-eucain.
Slower: Cocain, novocain, holocain.

Duration of Action.—This varies, mainly according to the rate of absorptive removal. It is of some practical importance for surface and subdural anesthesia, but it matters very little for infiltration anesthesia, for in this, the duration can be prolonged at will by the addition of epinephrin (except with tropacocain).

There are no experimental data on the duration of action after subdural injection. The corneal method, however, furnishes direct information for surface anesthesia. The time (in minutes) required for complete recovery after the application of the minimal concentrations producing complete anesthesia was (for hydrochlorids):

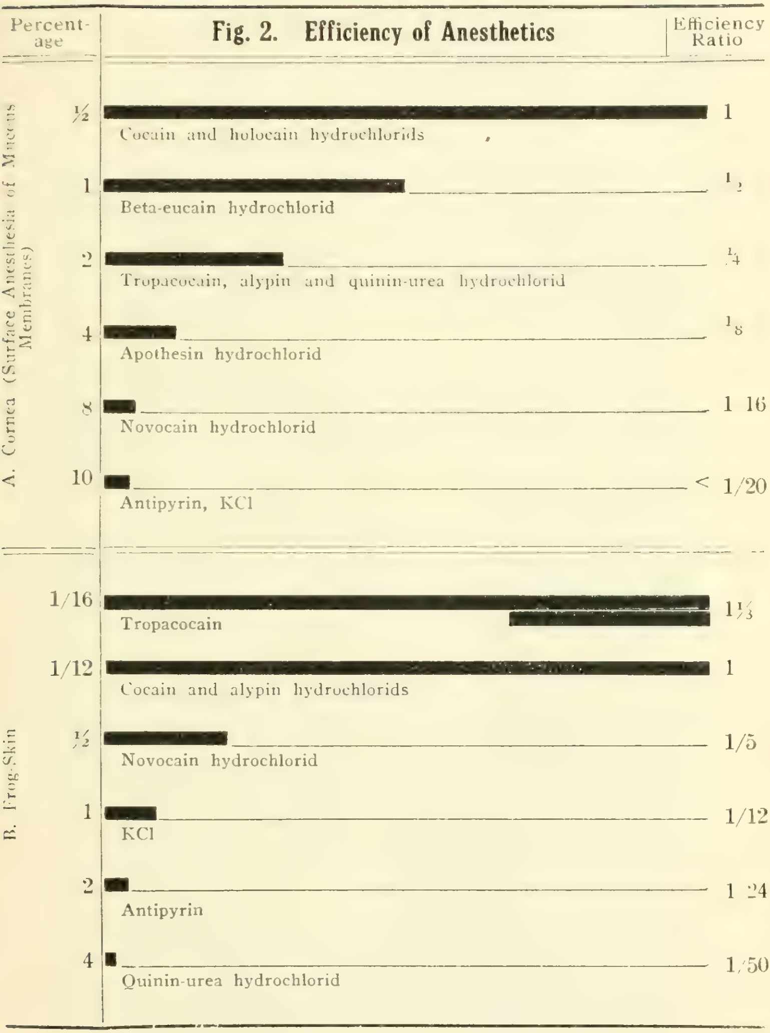

Fig. 2.—Efficiency of anesthetics in surface anesthesia.

From ten to fifteen minutes: cocain, tropacocain.

From twenty to thirty minutes: alypin, beta-eucain, holocain.

Fifty minutes: novocain.

Sixty-five minutes: quinin-urea.

THE EFFECT OF ALKALIZATION

O. Gros found that the addition of alkali increases very noticeably the efficiency of cocain and of the synthetic anesthetics, doubtless because the free anesthetic bases penetrate more readily than the salts. His results were based mainly on the motor fibers of nerve trunks.

My results confirm this potentiation for the motor-nerve trunk, and extend it to the sensory fibers of nerve trunks, to the frog-skin and to the cornea. On the other hand, no potentiation occurred on intracutaneous injection, evidently because this does not require penetration.

Accordingly, the addition of sodium bicarbonate, as suggested by Gros, serves no useful purpose for infiltration anesthesia. It may be of some use for intraneural and possibly for subdural injections, and it would be definitely useful for surface anesthesia. For these uses, the anesthetic should be made up in double concentration, and diluted, just before use, with an equal volume of 0.5 per cent. sodium bicarbonate. This increases the efficiency (for the cornea) as follows: Cocain, from one to two times; beta-eucain, two times; novocain, from two to four times; tropacocain or alypin, four times. For instance, a 0.5 per cent. solution of alypin in 0.25 per cent. sodium bicarbonate is just as effective as a 2 per cent. solution of alypin (the hydrochlorid) in plain saline.

This effects a considerable saving in anesthetic. It is not certain whether or not it modifies the toxicity.

Alkali should not be added to holocain or to quinin-urea.

EPINEPHRIN

This was introduced mainly by H. Braun, to prolong the local anesthetic action for infiltration and injection anesthesia. It is notably effective for this purpose. With intracutaneous injection, the anesthesia is prolonged for hours (tropacocain is an exception, since this destroys the vasoconstrictor action of epinephrin). The actual efficiency is not increased, so that there is no true synergism. The epinephrin acts solely by preventing the absorptive removal of the drugs. In practice, this means that the anesthesia can be maintained for the duration of an operation with much less drug, thus diminishing the chances of systemic toxicity, and obviating waste of anesthetic. The addition of epinephrin (about 1:50,000) is therefore advised for all injection anesthesias (except tropacocain).

On the other hand, epinephrin is quite useless for surface anesthesia; indeed, it rather diminishes the penetration and therefore the efficiency of the anesthetics on mucous membranes (cornea). I also doubt whether it would be of service in intraneural or subdural injections.

COMBINATIONS OF ANESTHETICS

The efficiency of mixtures of the local anesthetics corresponds to more or less complete summation, without any potentiation. In other words, if two solutions of different anesthetics are diluted until they are "just effective," then a mixture of the two solutions will also be "just effective"—no more and no less.

A single exception to this rule exists, namely, the potentiation of potassium with certain of the other anesthetics, discovered by Hoffmann and Kochmann. I have confirmed this for motor paralysis; but I cannot confirm it for sensory anesthesia. It has, therefore, no practical importance.

There would be little or no advantage in mixing the synthetic anesthetics with each other, except, perhaps, in mixing any of the synthetic anesthetics with quinin-urea, or with potassium chlorid. These are rather too feeble by themselves; but when they are used in non-irritant concentrations, being employed to take the place of a part of the synthetic anesthetics, the toxicity of the latter is correspondingly reduced.

SUMMARY

These investigations of the local anesthetics justify the following conclusions, as to their suitability for clinical uses, mainly from the standpoint of efficiency:

1. For the anesthesia of mucous membranes, the relative efficiency is shown in Figure *2A*. The numbers at the left show the percentages needed to equal 0.5 per cent. cocain hydrochlorid. Cocain, beta-eucain, alypin and tropacocain are the most useful; quinin-urea hydrochlorid is fairly active. Apothesin,[1] novocain and potassium chlorid are relatively inefficient. Alkalization increases the efficiency from two to four times. The solutions of the anesthetic salts may therefore be mixed with an equal volume of 0.5 per cent. sodium bicarbonate, without loss of efficiency, and with a saving of one half of the anesthetic. The mixtures, however, do not keep well, and should be recently made.

The addition of epinephrin does not increase the efficiency, and is probably useless.

2. For infiltration and injection anesthesia, the relative efficiency is shown in Figure 1*A*. Cocain, novocain, tropacocain and alypin are about equally efficient. Beta-eucain and quinin-urea hydrochlorids are intermediate; apothesin and potassium sulphate (or chlorid) are relatively inefficient.

The efficiency is not increased by alkalization. Epinephrin greatly prolongs the action, and should always be added (except to tropacocain).

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[1] Apothesin is a new proprietary anesthetic, said to be the cinnamic ester of gamma-diethylamino propyl alcohol hydrochlorid. It was obtained through the kindness of Dr. McCoy, U. S. Public Health Service.

The anesthetic action of potassium sulphate or chlorid is not great enough to be of real value.

A 1 per cent. (that is, isotonic solution) would be equivalent to about 0.125 per cent. of cocain or novocain. However, it may well be used in place of sodium chlorid for making anesthetic solutions, as suggested by Braun.

Several of the synthetic anesthetics can completely take the place of cocain. In view of this fact, it would be feasible to prohibit entirely the importation, manufacture, sale and use of the habit-forming cocain, except for scientific purposes.

REPORTING OF ACCIDENTS FROM LOCAL ANESTHETICS

(From the Journal A. M. A., Jan. 26, 1918, p. 258)

To the Editor: The Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association has undertaken a study of the accidents following the clinical use of local anesthetics, especially those following ordinary therapeutic doses. It is hoped that this study may lead to a better understanding of the cause of scuh accidents, and consequently to methods of avoiding them, or, at least, of treating them successfully when they occur.

It is becoming apparent that several of the local anesthetics, if not all of those in general use, are prone to cause death or symptoms of severe poisoning in a small percentage of those cases in which the dose used has been hitherto considered quite safe.

The infrequent iccurrence of these accidents and their production by relatively small doses point to a peculiar hypersensitiveness on the part of those in whom the accidents occur. The data necessary for a study of these accidents are at present wholly insufficient, especially since the symptoms described in most of the cases are quite different from those commonly observed in animals even after the administration of toxic, but not fatal, doses.

Such accidents are seldom reported in detail in the medical literature, partly because physicians and dentists fear that they may be held to blame should they report them, partly, perhaps, because they have failed to appreciate the importance of the matter from the standpoint of the protection of the public.

It is evident that a broader view should prevail, and that physicians should be informed regarding the conditions under which such accidents occur in order that they may be avoided. It is also evident that the best protection against such unjust accusations, and the best means of preventing such accidents consist in the publication of careful detailed records when they have occurred, with the attending circumstances. These should be reported in the medical or dental journals when possible; but when, for any reason, this seems undesirable, a confidential report may be filed with Dr. R. A. Hatcher, 414 East Twenty-Sixth Street, New York City, who has been appointed by the committee to collect this information.

If desired, such reports will be considered strictly confidential so far as the name of the patient and that of the medical attendant are concerned, and such information will be used solely as a means of studying the problem of toxicity of this class of agents, unless permission is given to use the name.

All available facts, both public and private, should be included in these reports, but the following data are especially to be desired in those cases in which more detailed reports cannot be made:

The age, sex, and general history of the patient should be given in as great detail as possible. The state of the nervous system appears to be of especial importance. The dosage employed should be stated as accurately as possible; also the concentration of the solution employed, the site of the injection (whether intramuscular, perineural or strictly subcutaneous), and whether applied to the mouth, nose, or other part of the body. The possibility of an injection having been made into a small vein during intramuscular injection or into the gums should be considered. In such cases the action begins almost at once, that is, within a few seconds.

The previous condition of the heart and respiration should be reported if possible; and, of course, the effects of the drug on the heart and respiration, as well as the duration of the symptoms, should be recorded. If antidotes are employed, their nature and dosage should be stated, together with the character and time of appearance of the effects induced by the antidotes. It is important to state whether antidotes were administered orally, or by subcutaneous, intramuscular, or intravenous injection, and the concentration in which such antidotes were used.

While such detailed information, together with any other available data are desirable, it is not to be understood that the inability to supply such details should prevent the publication of reports of poisoning, however meager the data, so long as accuracy is observed.

The committee urges on all anesthetists, surgeons, physicians and dentists the making of such reports as a public duty; it asks that they read this appeal with especial attention of the character of observations desired.

Therapeutic Research Committee of the Council
on Pharmacy and Chemistry of the American
Medical Association.

Torald Sollmann, Chairman,
R. A. Hatcher, Special Referee.

LOCAL HEMOSTATIC PROPERTIES OF COTARNINE AND SOME OTHER AGENTS*

PAUL J. HANZLIK

(From the Journal of Pharmacology and Experimental Therapeutics, January, 1918, p. 523)

From the Pharmacological Laboratory, Western Reserve University, Cleveland, Ohio.

Object of Investigation.—Claims have been made by K. Abel[1], Kehrer[2], Munk[3], Hulish[4], and Offergeld[5] that cotarnine hydrochloride (stypticin), cotarnine phthalate (styptol) and sodium phthalate act as efficient styptics or hemostatics both clinically and under experimental conditions. Their claims refer chiefly to the use of these drugs in uterine hemorrhages for which a certain degree of specificity is claimed (K. Abel), and also in connection with tooth extraction and hemophilia. Experimentally these claims have not been supported by the work of Laidlaw[6] on the coagulation of blood and blood pressure, and by Pilcher and Sollmann[7] on the vasomotor center (by direct stimulation). The literature of the manufacturers contains claims which appear exaggerated, and the experimental method used by Mohr and described by the gynecologist, K. Abel, is new and untried. It seemed worth while, therefore, to reinvestigate this class of preparations, particularly the salts of cotarnine, with the following objects in view:

1. Reliability of the method of K. Abel.
2. Repetition of the experiments of K. Abel.

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* This investigation was aided by a grant from the Therapeutic Research Committee of the Council of Pharmacy and Chemistry of the American Medical Association.

[1] Abel, K.: Berl. klin. Wchschr., 1905, No. 34.

[2] Kehrer: Monatsch. f. Geburtsh. u. Gynäk., 1907, No. 5, p. 709.

[3] Munk: Aertz. Centr. Az., 1899, No. 12.

[4] Hulish: Zahn. Rundschau, 1899, No. 431.

[5] Offergeld: Arch. f. Gynäk., 1915, No. 104.

[6] Laidlaw: Biochem. J., 1910, v, 243.

[7] Pilcher and Sollmann: J. Pharm. Exper. Therap., **6**: 391, 1915–1916.

3. Applications of other methods, which might suit the conditions and answer the claims that are made.

4. Comparison of the cotarnine salts with some well known and other hemostatics.

These will be discussed alone or together as appears suitable.

K. Abel's Method.—The description of this method by K. Abel[1] is as follows:

> If the skin on the ball of the foot (of a cat) be cut sufficiently deeply to expose the papillary layer, hemorrhage will ensue (which is spontaneously checked with difficulty, and which will recur merely on rubbing). If such a wound be touched with styptol or phthalate of ammonium the hemorrhage ceases after a short time and the wound assumes a pale red aspect, and it may be seen that the blood in the capillaries has coagulated.

The following description by Kehrer[2], while somewhat different, is essentially the same as K. Abel's and is cited because sodium phthalate was used in my experiments instead of the ammonium salt which apparently is unessential and possesses no specific properties:

> If a piece of skin is excised from the ball of the foot of a cat so that the papillary body is exposed, a hemorrhage will follow which only rarely ceases spontaneously and which can be increased by friction. If the solution of a neutral cotarnine salt or a neutral phthalate is applied to this wound, the hemorrhage will soon cease and the wound will appear pale red. The blood will clot in the capillaries themselves. If instead of a phalate another salt (NH_4Cl or ordinary salt) is employed the hemorrhage will not be checked, but on the contrary will become worse. The local hemostatic action of cotarnine phthalate (styptol) must therefore be regarded as specific for the cotarnine as well as the phthalic acid.

Limitations of the Method.—In my experiments the technic as described by K. Abel was carried out as closely and carefully as possible. On the whole it was impossible to make satisfactory preparations of the cat's pad. In order to secure bleeding for a suitable period of time it was necessary to incise the pad. Removal of the skin of the pad alone produced practically no bleeding. The procedure, therefore, of removal of the skin together with the deep papillary layer was adopted. This secured a free bleeding surface which continued to bleed for variable periods of time, though never indefinitely.

The bleeding appears to be of two kinds: (1) a capillary oozing from the papillary layer proper, and (2) venous from the venules underlying the skin and surrounding the edge of the wound. The bleeding from the venules is faster and more voluminous than from the papillae where it is slow and scanty. As a rule the veins continued to bleed irrespective of the application of drugs, pressure, or any other procedure during a certain interval, although after a sufficiently long interval the bleeding also stopped spontaneously. The oozing from the papillae could be diminished and stopped by pressure and certain drugs, but this was always difficult to judge on account of the contamination of the field with blood from the larger vessels, chiefly veins. Attempts were made to exclude the venous bleeding by continual mopping with pledgets of cotton, but the disturbance is so great and the success so limited that this practice was abandoned except with the larger veins.

Another limitation of the method is that continuous venous oozing removes the drug by "washing away." Repeated applications for this reason are necessary to insure continual contact of the drug with the wound.

The selection of a suitable control also proved difficult. The use of two pads from two different feet, one of which could serve as a control, and the other for treatment with drugs, proved unsuccessful because of the differences in the hemorrhages even with the most painstaking attempts to make the wounds alike in all respects. After some experimentation, a standard was found which could serve more or less efficiently for comparing the effects of other drugs.. Applications of the drug to be tested were made to the wound and after a sufficiently long interval, epinephrin (1:1,000), which I took as the standard, was applied. If the bleeding did not stop as soon as after the application of epinephrin, it was concluded that the drug was less efficient. Results obtained in this way, and by other methods are described in the protocols.

From all that has been said, it is apparent that the cat's pad method of testing hemostatics as used by Mohr and described by K. Abel is crude and far from ideal, even with the use of the slight modification that I have introduced. The results are presented for whatever they are worth. Briefly, it may be stated that the results obtained were enirely negative for stypticin, styptol and sodium phthalate, but that epinephrin (1:1,000) can stop bleeding temporarily and more or less efficiently. In all, experiments on eight cats' paws were performed, in some cases the pads having been treated repeatedly. The discussion of the results follows:

TABLE 1. EFFECT OF HEMOSTATICS ON BLEEDING FROM CAT'S PAD*

Treatment	Hemorrhage			Number of Applications	Remarks
	Stoppage Lasted, Min.	Stopped at End of, Min	Continued at End of, Min.		
Untreated	83	..	..	......	Superficial wound
	8				
Epinephrin 1:1,000	1	19	..	2 (drops)	
	3	5	..	Irrigation	
	2	41	..	Several irrigations	
	3	7½	..	Several irrigations	
Stypticin 10 per cent.	..	..	21½	7	
	..	..	18	3	
	..	..	10	2	
Stypticin (dry) after epinephrin	..	..	7 (oozing)		
Styptol 10 per cent.	..	..	6	......	Several irrigations
	.	..	19	7	
Styptol (dry)	..	..	10	2	
Styptol (dry) after epinephrin	..	..	7		
Sodium phthalate 10 per cent.	..	..	4	Continual bath	
	..	..	10	3	
Tannin 1 per cent.	..	..	16	4	
Tannin 10 per cent.	..	..	11½	Several	
Tannin (dry)	..	..	10		
Hydrastinin 10 per cent.	..	..	5	2	

* The wound was about 1 cm. by 1 cm.

Bleeding from the Untreated Cat's Pad.—The pad was incised according to the procedure described, and noting the time interval during which spontaneous stoppage of bleeding took place. The blood was mopped from time to time with a pledget of cotton. The following results were obtained: 8 minutes, 13½ minutes, and 1 hour and 23 minutes, respectively. This shows the extreme variability of time within which cessation of bleeding can occur spontaneously, and obviously is a serious obstacle to judgment concerning the effects of hemostatics without some suitable standard.

Comparative Effects of Cotarnine Salts and Other Agents on Bleeding from the Cat's Pad.—In the first series of experiments, the results of which are presented in Table 1, various drugs, in solution and dry form, were applied directly to the wound by irrigation and dusting, respectively. It is seen that the application of epinephrin (1 : 1,000) stopped hemorrhage in about 3 minutes and the stoppage lasted from 5 to 44 minutes. Almost immediately after the application of epinephrin, the wound appeared paler and gradually the oozing of blood stopped, though variably owing to contamination with venous hemorrhage. On the other hand, oozing continued uninterruptedly and unchanged at the end of from 4 to about 22 minutes (average 10 minutes) after the application of stypticin, styptol, sodium phthalate and tannin either in solution or dry form, and in spite of repeated applications. In fact, following the application of styptol and stypticin the hemorrhage appeared to be increased somewhat. Objection might be raised against the method of application, namely, that sufficient drug is not retained in contact with the wound in account of the continuous oozing from the venules which removes it by "washing away" and also dilutes it.

In the following series, therefore, the wounds were always mopped first, then irrigated with the solution and, in addition, a pledget of cotton placed over this was maintained saturated with the solution. When the powdered drug was used, this was reapplied from time to time and held in place by a pledget of cotton. The results of the experiments have been summarized in Table 2, and are, in general, confirmatory of those in Table 1.

Epinephrin (1 : 1,000) partially stopped the hemorrhage in 2 to 3 minutes and practically completely in 5 to 12 minutes after application. The stoppage lasted from 11 to 20 minutes. Powdered epinephrin tablets (containing 1 mgm.) of epinephrin appeared to be less efficient, for it is undoubtedly necessary for the epinephrin to be in solution before it can act. Hemorrhage continued freely at the end of 22 to 42 minutes after the application of styptol, stypticin and sodium phthalate. In fact, styptol seemed to increase the hemorrhage. Kephalin slowed the oozing in 22 to 44 minutes after application, but in no case was there complete stoppage.

TABLE 2.—EFFECT OF HEMOSTATICS ON BLEEDING FROM CAT'S PAD

	Hemorrhage			
Treatment	Stopped at End of, Min.	Stoppage Lasted, Min.	Continued at End of, Min.	Mode of Application
Epinephrin 1:1,000	2 (partial)	..	..	Pledget of cotton
	12 (complete)	20	..	Pledget of cotton
	2 (partial)	..	..	Pledget of cotton
	7 (complete)	..	..	Pledget of cotton
	3	20	..	Pledget of cotton
	4 (partial)	..	..	Pledget of cotton
	12 (complete)	..	..	Pledget of cotton
	3 (partial)	..	..	Pledget of cotton
	8 (complete)	..	..	Pledget of cotton
	3	..	..	Pledget of cotton
	3 (partial)	..	..	Pledget of cotton
	5 (complete)	16	..	Pledget of cotton
	5 (complete)	13	..	Pledget of cotton
	3 (partial)	..	..	Pledget of cotton
	5 (complete)	12	..	Pledget of cotton
	6	11	..	Pledget of cotton
Stypticin 10 per cent		..	22	Pledget of cotton
			38	Pledget of cotton
Stypticin (dry)		..	31	Pledget of cotton
			42	Pledget of cotton
Styptol 10 per cent		..	22	Pledget of cotton
			36	Pledget of cotton
Styptol (dry)		..	29	Pledget of cotton
			40	Pledget of cotton
Sodium phthalate 10 per cent		..	23	Pledget of cotton
Kephalin[*] 5 per cent	(Slowed) 44			
	22 (complete)			
	44 (complete)			
	22 (complete)			

[*] Armour's product, 5 per cent. emulsion, in saline, was used.

It may be concluded that styptol, stypticin and sodium phthalate (in strong solution or dry form) are inefficient as hemostatics for superficial wounds of the cat's pad as compared with a much weaker solution of epinephrin (1:1,000), which is promptly effective though the effects are temporary and also somewhat variable. The experiments with tannin (strong solution and dry form), hydrastinin and kephalin are too incomplete to permit the drawing of definite conclusions, but it appears that their hemostatic efficiency under the conditions they were used here is comparatively limited.

Experiments on the Cock's Comb.—Applications of the various hemostatics were made to (1) superficially denuded areas (about 0.5 to 1 cm. by 0.5 to 1 cm.) of the comb, and (2) to wounds made by section across the tip of the comb. The hemorrhage from the denuded areas was slow and occurring at discrete points in the white fibrous portion of the organ. More bleeding took place from the edges of the wound. The hemorrhage across the tip was freer and more voluminous, resembling the venous type. The bleeding from this did not stop for a very long time, while in the untreated denuded areas the bleeding stopped of its own accord in one hour. The following results (Table 3) were obtained with the various drugs.

Epinephrin (1:1,000) stopped the bleeding in two minutes and this lasted for six to ten minutes. Powdered epinephrin tablets stopped it in three minutes and this lasted thirty-five minutes. On the other hand, hemorrhage did not stop at the end of thirty-three to seventy-four minutes after repeated applications of stypticin 10 per cent, styptol 10 per cent, sodium phthalate 10 per cent, glycerite of tannic acid (U. S. P.) and powdered stypticin. In fact, after stypticin and styptol the oozing seemed to be increased in the same way as in the experiments on cats' pads. The results, in general, are confirmative of those described in the previous section.

It may be concluded that the local application of epinephrin promptly and effectively though temporarily stops bleeding from superficial wounds of the cock's comb, while stypticin, styptol and sodium phthalate are ineffective.

Effects on Liver and Spleen. 1. Excised organs. Freshly excised organs of the cat were used. The results are presented in Table 4. Direct application of a powdered epinephrin tablet over an area of about 0.5 cm. by 0.5 cm. to the liver and spleen caused local blanching in two minutes and this lasted for about five minutes. At the end of this time the whitening effect disappeared, the site of application again resuming a dusky brown appearance, and slight oozing of blood

TABLE 3.—EFFECT OF HEMOSTATICS ON COCK'S COMB*

	Hemorrhage				
Treatment	Stopped at End of, Min.	Stoppage Lasted, Min.	Unchanged at End of, Min.	Number of Applications	Remarks
Untreated..........	60				
Epinephrin 1:1,000	2	10	About 15; stronger at 30	3	
	2	6	....	1 c.c. irrigation	
	..	..	33	Continuous to wound across comb	
Epinephrin (dry) (1 mg. tablet)	3	35			
Stypticin 10 per cent.	..	..	74	Irrigation and saturated cotton pledget for 60 minutes to wound	Bleeding increased
Stypticin (dry)....	..	..	37	Repeated dusting on wound	Bleeding increased
Styptol 10 per cent.	..	..	74	Irrigation and saturated cotton pledget for 60 minutes to wound	Bleeding increased
Sodium phthalate 10 per cent.	..	..	37	Saturated pledget of cotton on wound	
Glycerite of tannic acid (U. S. P.)	..	..	35	Seven to wound across comb	

* The results from both superficial wounds and across the comb are embodied in the table.

continued. On the other hand, applications of powdered styptol and stypticin produced no change in the appearance of both the liver and the spleen, except for the brownish or straw color stain left after the use of styptol, this being the natural color of its solution. This holds true for all the experiments thus far and subsequently to be described.

2. Intact liver. The application of 1 : 1,000 epinephrin solution to an abrazed area (about 0.5 cm. by 0.5 cm.) had no effect when the oozing was rapid and voluminous. When the oozing was slow and not so voluminous, stoppage was produced almost immediately after application, and there was blanching of about the same kind and extent as in the excised organ. The following drugs, when applied in the same way as epinephrin, produced no visible effects on the bleeding; powdered styptol and stypticin, 10 per cent. styptol and stypticin, 5 per cent. kephalin, glycerite of tannic acid (U. S. P.) and lung extract (1 cc. of which was equivalent to 1 gram of the moist freshly excised organ of the dog).[8]

Uterus.—The uterus of the guinea-pig does not appear to be a satisfactory test object. The nonpregnant organ does not bleed sufficiently, bleeding being impeded by the contraction of the cut muscles, and in the pregnant organ, the observations are impaired by movement of the fetus and bulging of amnion, which because of the pressure produced tend to stop bleeding naturally. However, a few observations were made on the nonpregnant and pregnant organ of the guinea-pig. The data from these are presented in Table 4.

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[8] Fischl (Arch. Kindhkl., 1916, **65**: 188) has made the claim that lung extract is an excellent local hemostatic, the effects being so striking that a patent has been applied for. The strength of Fischl's extract is unknown to me. Fischl's claim would not gain support from a prior claim of Gutmann's (Compt. rend. soc. d. biol., **76**: 349, 1914), namely, that lung extract diminishes the coagulability of blood.

TABLE 4.—EFFECT OF HEMOSTATICS ON VISCERA

Treatment	Hemorrhage Stopped at End of, Min.	 Stoppage Lasted, Min.	 Continued at End of, Min.	Remarks
		Intact Liver of Cat		
Epinephrin 1:1,000..	2	..	....	Blanching
	None	..	....	Oozing rapid
	Immediate	..	....	Oozing slow
Epinephrin (dry)....	..	..	....	Oozing slow
Styptol 10 per cent. .	..	..	10	No blanching
			15	Oozing increased
Styptol (dry)........	..	..	13	
Stypticin 10 per cent.	..	..	Continuous	
Stypticin (dry)......	..	..	14	
Sodium phthalate 10 per cent..........	..	..	10	No blanching
Kephalin 5 per cent.	..	..	Continuous	
Glycerite of tannic acid (U. S. P.).....	..	..	Continuous	
		Intact Tongue of Cat		
Epinephrin 1:1,000...	..	..	5	No blanching
Styptol 10 per cent. .	..	..	5	No blanching
		Freshly Excised Liver of Cat		
Epinephrin (dry)....	Blanching only 2	4	Blanching only	
Styptol (dry)........	..	..	No change during the afternoon	
Stypticin (dry)......	..	..	No change during the afternoon	
		Freshly Excised Spleen of Cat		
Epinephrin (dry)....	2	5		
Styptol (dry)........	..	..	No change during the afternoon	
Stypticin (dry)......	..	..	No change during the afternoon	
		Intact Nonpregnant Uterus of Guinea-Pig		
Epinephrin (dry)....	1	7½	....	Blanching only at edge of wound
Epinephrin (1:1,000).	1	8	....	Wound pale
Stypticin (dry)......	..	..	Continuous bleeding end of 18 minutes	No blanching; bleeding increased
Stypticin 10 per cent.	..	..	Continuous bleeding end of 18 minutes	No blanching; bleeding increased

TABLE 4. EFFECT OF HEMOSTATICS ON VISCERA Continued

Intact Pregnant Uterus of Guinea-Pig

Epinephrin (dry)....	8	8	....	Blanching only at edges of wound
Epinephrin (1:1,000).	1	10	....	Wound blanched
Stypticin (dry)......	..	..	Bleeding continued end of 17 minutes	When embryo moves and bulges amnion into wound, bleeding stops; no blanching
Stypticin 10 per cent.	..	..	Bleeding continous	No blanching; oozing increased

Intact Pregnant Uterus of Guinea-Pig (No wounds; direct application of drugs to organ)

Epinephrin (dry)	Blanching in 1 minute; disappeared at end of 9 minutes after application
Stypticin (dry)	In 2 minutes, uterus became redder; 4 minutes later organ very red, organ injected with blood, redness spreading beyond place of application of stypticin; in 6 minutes, organ is "angry-looking," very red, vessels are tortuous and stand out prominently. At end of 20 minutes after application redness lessened, vessels were less tortuous. At end of 30 minutes organ appears about same as before application of stypticin

Pregnant Uterus.—When a pulverized epinephrin tablet containing 1 mg. of epinephrin was placed on the organ, blanching occurred in one minute, and this lasted for nine minutes. On the other hand, when dry stypticin was applied, the organ appeared more turgid with blood and the vessels were more tortuous. In other words, a rather marked hyperemia occurred which spread beyond the place of application of the drug, and this lasted for about half an hour.

The application of dry epinephrin to a bleeding abrasion resulted in reduction of the hemorrhage in four minutes, and complete stoppage took place in eight minutes. After the application of dry stypticin to a similar wound, the hemorrhage was markedly increased and continued so at the end of seventeen minutes. When the fetus moved, and the amnion bulged into the wound, the bleeding stopped momentarily, but resumed again when the pressure was removed.

Epinephrin 1 : 1,000 solution, stopped bleeding in one minute and this lasted for about ten minutes, while the application of 10 per cent stypticin caused the bleeding to increase.

Nonpregnant Uterus.—The results were similar to those with the pregnant organ. Epinephrin, in dry form (1 mg. tablet) and solution (1 : 1,000), stopped bleeding in one minute and this lasted for about seven minutes. Stypticin in dry form and solution (10 per cent.) caused an increase in the bleeding.

The results obtained with the uterus are, therefore, confirmative of those obtained with the other viscera. Uterine vessels were perfused, and this will be referred to presently.

Peripheral Vasoconstriction in the Rabbit's Ear.—There is no reason to believe that epinephrin, as used in the experiments described, acts as a hemostatic in any other way than by local vasoconstriction. Whether it involves both the capillaries and arterioles or only the one or the other is immaterial here. The fact that it was effective is indicative of its absorption. It may be that the cotarnin salts (stypticin and styptol) as well as the other agents can also act by vasoconstriction, and the objection might be raised that they did not exert any such action here because they were either not absorbed or only incompletely so and thereby prevented from exerting their action on the vessels. Injections of the various agents, therefore, were made into the base of the rabbit's ear in the vicinity and around a large vessel according to the method recently described by Meltzer and Auer.[9]

With epinephrin (1 : 1,000), blanching begins to take place in less than one minute and practically the entire ear is white at the end of one to three minutes, and cold to the touch. This persists for several hours, about seven to twenty-four. The effects are very striking and were repeatedy obtained. This is confirmative of Meltzer and Auer.

In the first series (Part A of Table 5) of experiments, one ear was used for the epinephrin control and the other ear was treated with the drug to be tested. Following the injection of styptol (10 per cent.) and stypticin (10 per cent.), the ears remained warm and no blanching was visible at the end of seventeen to sixty-four minutes. The epinephrin controls in each case responded in the usual manner. The styptol and stypticin ears remained unchanged the entire afternoon (four hours).

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[9] Meltzer and Auer: Proc. Soc. Exper. Biol. Med., **14**: 54, 1916.

In another series of experiments (Part B of Table 5), the ears were first treated with the different agents and, after a given lapse of time, epinephrin was injected into the same place where the drug to be tested was injected. The results obtained were as follows:

Styptol (10 per cent.).—No blanching was observed and the ears remained warm at the end of one hour and fifteen minutes and one hour and seventeen minutes, respectively, after injection. Injection of epinephrin (1 : 1,000) into the same ears, and in the same place where the styptol was injected, produced blanching and cooling within one minute after injection.

Stypticin (10 per cent.).—No blanching was observed and the ears remained warm at the end of one hour and five minutes and one hour and nine minutes, respectively, after injection. At the end of this time, injection of 1 : 1,000 epinephrin into the stypticin areas of the same ears was made with the result that marked blanching and a sensation of coolness to the touch throughout the ear occurred within one minute.

Other Agents.—Lung extract and kephalin (5 per cent.) produced no effects whatsoever after forty-nine and fifty-two and twenty-two minutes, respectively, whereas injection of 1 : 1,000 epinephrin in the previously injected areas of the same ears gave the usual responses of blanching and cooling within about one minute. Following the injection of hydrastinin (10 per cent.) a small area of paleness surrounded the blood vessel around which the injection was made in twenty-two minutes; the remainder of the ear and the portion supplied by the vessel exhibited no unusual changes. Injection of 1 : 1,000 epinephrin here again promptly produced the usual responses within one minute.

It may be concluded that epinephrin in weak solution (1 : 1,000) acts as a prompt and powerful vasoconstrictor when injected into the base of the rabbit's ear.

TABLE 5. EFFECT OF HEMOSTATICS ON VESSELS IN RABBIT'S EAR

Drug Injected	Condition of Ear (Paling or White Denotes Vasoconstriction)	Temperature of Ear	Remarks
	Part A		
Epinephrin (1:1,000)	Begins to pale in less than ½ minute	Cool	Nicotinized rabbit
	Begins to pale in less than 1 minute; completely white in 3 minutes	Cold	Normal rabbit
	Beginning paling in less than 1 minute; completely white in 3 min.	Cold	Normal rabbit
Stypticin 10 per cent.	No paling end of 27 minutes	Slightly cool	Nicotinized rabbit
	No paling end of 64 minutes	Warm	Normal rabbit
Styptol 10 per cent.	No paling end of 28 minutes	Warm	Nicotinized rabbit
	No paling end of 17 minutes	Warm	Normal rabbit
	Part B		
Styptol 10 per cent.	Ear unchanged; no paling end of 1 hour and 15 minutes	Warm	Normal rabbit
Epinephrin 0.1 per cent. in site of styptol injection	Beginning paling within 3 seconds; ear completely white in 1 minute	Cold	Normal rabbit
Styptol 10 per cent.	Ear unchanged; no paling end of 1 hour and 17 minutes	Warm	Normal rabbit
Epinephrin 0.1 per cent. in site of styptol injection	Beginning paling in less than ½ minute; completely white in 1 minute	Cold	Normal rabbit
Stypticin 10 per cent.	Ear unchanged; no paling end of 1 hour and 5 minutes	Warm	Normal rabbit
Epinephrin 0.1 per cent. in site of stypticin injection	Paling in 2 seconds; ear completely white in 2 minutes	Cold	Normal rabbit
Stypticin 10 per cent.	Ear unchanged; no paling end of 1 hour and 9 minutes	Warm	Normal rabbit
Epinephrin 0.1 per cent. in site of styptol injection	Ear completely white in 1 minute	Cold	Normal rabbit
Lung extract	Ear unchanged; no paling end of 49 minutes	Warm	Normal rabbit
Epinephrin 0.1 per cent. in site of lung extract injection	Ear white in 2 minutes	Cold	Normal rabbit
Lung extract	Ear unchanged; no paling end of 52 minutes	Warm	Normal rabbit
Epinephrin 0.1 per cent. in site of lung extract injection	Ear white in 1 minute	Cold	Normal rabbit
Kephalin 5 per cent.	Ear unchanged; no paling in 22 minutes	Warm	Normal rabbit
Epinephrin 0.1 per cent. in site of kephalin injection	Ear white in 1 minute	Cold	Normal rabbit
Hydrastinin 10 per cent.	Paling around vessel injected in 22 minutes	Warm	Normal rabbit
Epinephrin 0.1 per cent. in site of hydrastinin injection	Ear white in 1 minute	Cold	Normal rabbit

Strong solutions (10 per cent.) of stypticin and styptol when administered under the same conditions as epinephrin produce no such demonstrable vascular effects. The injection of epinephrin after the injection of styptol or stypticin into the same ear promptly produces the usual vasoconstrictor effects as in the untreated ear, showing that the blood vessels are responsive and not affected by previous treatment with strong solutions of stypticin and styptol.

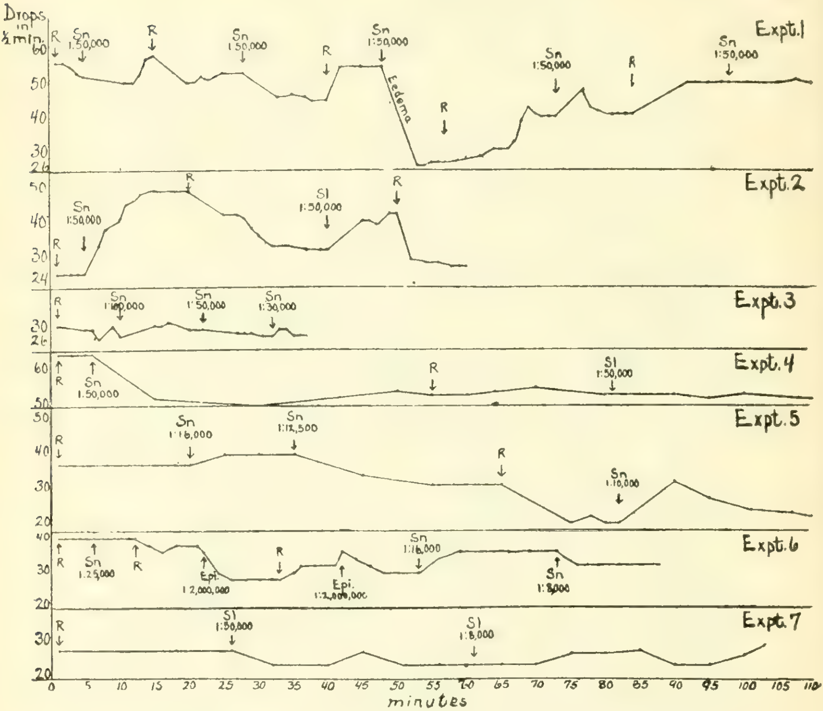

FIG. 1.—EFFECT OF COTARNIN SALTS ON FROG'S VESSELS.

The abbreviations have meanings as follows: *R.*, frog's Ringer solution; *Sn.*, stypicin (cotarnin hydrochlorid); *Sl.*, styptol (cotarnin phthalalte); *Epi.*, epinephrin. All drugs were dissolved in frog's Ringer solution.

Perfusion of Frog's Vessels.—This was done in the usual way by the Läwen-Trendelenburg method. The results obtained are presented in Figure 1.

Stypticin.—Sixteen applications of stypticin in concentrations varying from 1 : 100,000 (0.001 per cent) to 1 : 10,000 (0.01 per cent.) were made in six different experiments. The results are somewhat variable. The majority (50 per cent.) indicate definite relaxation of the vessels, and of the remaining about 38 per cent. showed varying degrees of constriction. About 12 per cent. of all the applications showed no change. This variability may be accounted for by the presence of edema in some of the experiments as a complicating factor.

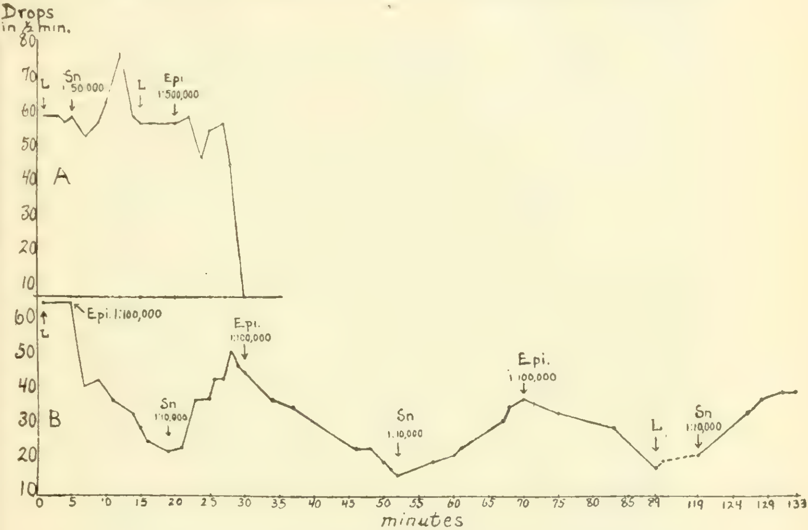

FIG. 2.—EFFECT OF COTARNIN HYDROCHLORID (STYPTICIN) AND EPINEPHRIN ON UTERINE VESSELS.

The abbreviations have meanings as follows: *Sn.*, stypticin; *Epi.*, epinephrin; *L.*, Locke's solution. The drugs were dissolved in Locke's solution.

Styptol.—The majority of four observations made with styptol in concentrations ranging from 1 : 50,000 (0.002 per cent.) to 1 : 8,000 (0.0125 per cent.) indicate a definite vascular dilatation.

In this connection, it may be added that similar effects were observed on the perfused frog's heart (Straub method). Of the two salts, styptol (cotarnin phthalate) was more depressing than stypticin (cotarnin hydrochlorid). It is obvious that these results would have only an indirect bearing on hemostasis. In some experiments, stypticin produced a moderate increase in pulse rate with depression of or no change in amplitude and tonus.

From all this it seems permissible to conclude that the effects of stypticin and styptol on the peripheral vessels of the frog are unfavorable to the claims made for their local hemostatic priperties.

Perfusion of Uterine Vessels.—The cornua of a rabbit's uterus were perfused (at room temperature) separately according to the Läwen-Trendelenburg technic. The results are presented in Figure 2. From curve A, which represents perfusion of the vessels of the right cornuum, it is seen that stypticin produced dilatation, while epinephrin (used as a control) caused constriction. The results in curve B, which represents perfusion of the left cornuum, show that stypticin relaxes vessels previously constricted by epinephrin. The results are similar to those obtained on frog's vessels, and confirm also the observations made with direct application of stypticin to the uterus.

It is justifiable to conclude that the effects of stypticin (cotarnin hydrochlorid) on uterine vessels are not conducive to hemostatic efficiency. In fact quite the contrary would be expected, that is, an increase in hemorrhage.

SUMMARY

1. Epinephrin (1 : 1,000 and powdered tablets containing 1 mg.) acts as an efficient hemostatic for small and superficial wounds. The effects are temporary and somewhat variable. It seems to be ineffective for stopping venous hemorrhage.

2. Stypticin and styptol (10 per cent. solutions and dry powders) are inefficient as local hemostatics, even after long periods of and repeated application. In

fact, hemorrhage tends to be somewhat aggravated after the application of these agents, and styptol is irritating.

3. Epinephrin (1 : 1,000) is a prompt and effective peripheral vasoconstrictor in the rabbit's ear, while styptol and stypticin (10 per cent. solutions) are inactive and inefficient. Styptol and stypticin produce relaxation of frog's vessels, and stypticin of uterine vessels.

4. The following agents do not appear to act either as local hemostatics or peripheral vasoconstrictors; sodium phthalate 10 per cent, lung extract 100 per cent, kephalin 5 per cent, and hydrastinin hydrochlorid 10 per cent. With the exception of the phthalate, however, final conclusions are reserved until experiments with these drugs have been extended.

CONCLUSIONS

The evidences obtained, it is believed, justify the following conclusions regarding the original objects of the investigation.

1. The original method described by K. Abel is at best a crude method, rendering positive deductions from results obtained with it somewhat hazardous unless fortified by other methods of observation.

2. The claims made by K. Abel that stypticin and styptol act as hemostatics on the incised pad of the cat's paw have not been confirmed.

3. Other methods that were used for testing out the alleged hemostatic properties of stypticin and styptol also failed to demonstrate any such effects.

4. As compared with epinephrin, the salts of cotarnin (styptol and stypticin) and sodium phthalate are certainly inferior hemostatics, and probably entirely inactive in this respect. This conclusion is fully confirmed by results which I have since obtained with a quantitative method for the study of local hemorrhage, and shortly to be reported.

COMPARATIVE ACTIVITY OF LOCAL ANESTHETICS

II. PARALYSIS OF SENSORY NERVE FIBERS *

TORALD SOLLMANN
CLEVELAND

(*From the Journal of Pharmacology and Experimental Therapeutics, February, 1918, p. 1.*)

I. INTRODUCTION.

The efficiency of local anesthetics on the sensory fibers of nerve trunks is of greater practical importance than their efficiency on the motor fibers. However, it can be measured in animals only by the transmission of reflexes, which introduces complicating factors.

This principle was first used on mammals by Pototzky,[1] 1903, under Biberfeld. He exposed the sciatic or brachial nerve of rabbits (sometimes dogs), for some distance, sprinkled it with the anesthetic in substance: and repeated the stimulation in five minute intervals, noting the time of paralysis. The method was not quantitative since the concentration of the aneshetic was unknown.

The method was again tried by Fromherz,[2] 1914. However, he employed solutions, and instead of determining the time of paralysis, which is variable, he determined the concentration required for complete anesthesia. His animals had received morphin, 10 to 15 mg. per kilogram of body weight. He applied the method to phenyl urethane derivatives, which are without interest in this connection.

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* From the Pharmacological Laboratory of the School of Medicine of Western Reserve University.

* This investigation is partly supported by a grant from the Committee on Research of the Council on Pharmacy and Chemistry of the American Medical Association.

1. Pototzky, C.: Arch. internat. Pharmacod., **12**: 129, 1903.
2. Fromherz, K.: Arch. exper. Path. u. Pharmakol., **76**: 257, 1914.

He also made some experiments with novocain and cocain, and concluded that much lower concentrations are effective than those required when these anesthetics are applied to the frog's foot or the cornea. For instance, he states that the minimal effective concentrations for rabbits are:

	Sciatic, per Cent.	Cornea, per Cent.
Novocain	0.0156	2
Cocain	0.01	1

No investigation appears to have been published in which the method had been applied systematically to frogs, as in the present work.

Technic of Experiments on Paralysis of Reflexes (Sensory Paralysis) by Application to the Nerve Trunk (Sciatic).—The frogs were decapitated, leaving the lower jaw. A transverse section was made through the abdomen just below the sternum, comprising about two-thirds of the circumference. The viscera were removed through this opening. The frogs were suspended by the lower jaw, and the eviscerated body cavity, which formed a little pocket, was filled with pledgets if cotton saturated with the anesthetic solution, and then a further quantity of the solution was poured in, to insure thorough saturation. About 4 c.c. of solution were applied in this way. The drugs were dissolved in eighth-molecular sodium chlorid solution.

At intervals the persistence of the reflex path was tested by immersing the feet in tenth-normal hydrochlorin acid. The legs were rinsed after each application.

When the reflexes had disappeared, or some time later, the excitability of the motor fibers of the sciatic nerve was tested by electric stimulation of the plexus. This was successful in every case. Paralysis must therefore have been due to the sensory fibers of the sciatic, or to the dying of the central nervous system. The results indicate that paralysis inside of thirty minutes or so is due to paralysis of the nerve fibers. After thirty to forty minutes, the paralysis might well be due to the death of the reflex centers, and therefore can not be counted. Altogether, the results are not as reliable as those of direct immersion of the motor trunk; but they give sufficient approximations for many practical purposes.

The results cannot be compared directly with the immersion of the entire nerve by the method used for motor fibers. The latter evidently secures better penetration and therefore requires lower concentrations.

II. RELATIVE EFFICIENCY OF THE ANESTHETICS ON SENSORY FIBERS.

The mean results are shown in Table 1, and more conveniently in Table 2.

The efficiency ratios for sensory and motor fibers are compared in Table 3.

Table 3 shows that cocain, novocain and tropacocain with direct immersion have the same ratio of efficiency on sensory and on motor fibers; but that potassium, alypin, quinin and especially antipyrin are relatively less efficient for sensory fibers.

One per cent $NaHCO_3$ and eighth-molecular NaCl or Na_2HPO_4 appear to have a weak paralytic effect, but not sufficient to be quite certain.

III. EFFECTS OF ALKALI

In these determinations, ½ per cent of sodium bicarbonate was added to the anesthetics. The mean results are shown in Table 4. According to the last column of the table, the increase of efficiency by alkalization is about two to eight times. This is marked, but only about one-half as much as with the motor fibers (compare Table 5 of the first paper of this series).

IV. EFFECT OF EPINEPHRIN

The addition of epinephrin (1 : 10,000) to cocain or novocain hydrochlorids, ¼ and ⅛ per cent, does not alter the time of paralysis. Epinephrin, therefore, does not directly increase the paralytic action of cocain or novocain on sensory fibers.

TABLE 1.—TIME OF COMPLETE SENSORY PARALYSIS OF SCIATIC TRUNK (MEDIAN VALUES)

Per Cent. of Drug	Cocain hydrochlorid	Novocain hydrochlorid	Tropacocain hydrochlorid	Alypin hydrochlorid	KCl	Quinin urea hydrochlorid	Antipyrin (in water)	NaCl M/8	NaHCO$_3$	Na$_2$HPO$_4$ M/8
8	......	......	......	......	......	......	30 × 50	......	......	......
4	......	......	......	......	......	......	40 × 80	......	......	......
2	......	......	......	......	......	20 × 30	40 × 60	......	......	......
1	5	......	......	5 × 10	5 × 10	60 ×	× 60	20 × 40	20 × 40	20×40
1/2	5 × 10	10 × 15	5 × 10	10 × 20	20 × 40	......	......	......	......	......
1/4	20 × 30	20×40	20 × 40	30 × 50	50 ×	......	......	..	. .	
1/8	30 × 50	50 ×	40 × 80	......	......	......	......	......	......	......

In this and in all other tables, the numbers to the left of the × denote the time (minutes) when there was no paralysis; and the numbers to the right of the × denote the time (minutes) when paralysis was completed.

TABLE 2.—CONCENTRATIONS PRODUCING PARALYSIS OF SENSORY FIBERS OF FROG'S SCIATIC IN A GIVEN TIME

	Time of Sensory Paralysis					Ratio of Efficiency (Paralytic Concentrations) in Terms of Cocain Hydrochlorid = 1				
Minutes...........	0-5	5-10	10-20	20-40	>40	5-10	10-20	20-40	>40	
	Percentage					Ratio				Mean
Drugs										
Cocain hydrochlorid..........	1	1/2	..	1/4	1/8	1	1	1	1	1
Novocain hydrochlorid..........	..	..	1/2	1/4	1/8	..	<1	1	1	1
Tropacocain hydrochlorid.......	..	1/2	..	1/4	1/8	1	1	1	1	1
Alypin hydrochlorid..........	..	1	1/2	..	1/4	1/2	<1	<1	1/2	1/2
KCl................	..	1	..	1/2	1/4	1/2	1/2	1/2	1/2	1/2
Quinin urea hydrochlorid.......	..	..	..	2	1	..	..	1/8	1/8	1/8
Antipyrin (in water)..........	..	..	..	8	4	..	..	1/32	1/32	1/32

TABLE 3.—EFFICIENCY RATIOS

	Motor Fibers	Sensory Fibers
Cocain hydrochlorid...... Novocain hydrochlorid...... Tropacocain hydrochlorid......	1	1
KCl......	1	1/2
Alypin hydrochlorid......	3/4	1/2
Quinin urea hydrochlorid......	1/5	1/8
Antipyrin......	1/8	1/32

TABLE 4.—EFFECTS OF ALKALI ON SENSORY FIBERS OF FROG'S SCIATIC. COMPARISON OF MEAN TIME OF PARALYSIS WITH THE HYDROCHLORIDS AND BASES

	Per Cent. of Anesthetic					Efficiency Ratio*
	1	½	¼	⅛	1/16	
Cocain hydrochlorid..........	<5	5×10	20×30	30×50		
Cocain base..................	......	<5	10×20	10×20	20×40	2 to 4
Novocain hydrochlorid........	......	10×15	20×40	>50		
Novocain base................	......	5×10	<5	10×20	>60	2 to 4
Tropacocain hydrochlorid.....	......	5×10	20×40	40×80		
Tropacocain base.............	......	<5	<5	5×10	>60	2 to 4
Alypin hydrochlorid..	5×10	10×20	30×50			
Alypin base..................	......	<5	<5	30×50	....	2 to 8

* The efficiency ratio as judged by the concentration required to produce paralysis in a given time, the base being × times as efficient as the hydrochlorid.

V. MIXTURES OF COCAIN WITH NOVOCAIN OR QUININ UREA HYDROCHLORIDS

The sensory fibers, just like the motor fibers, gave simple summation without potentiation, as shown in Table 5.

TABLE 5.—MIXTURES OF COCAIN WITH NOVOCAIN AND QUININ UREA HYDROCHLORIDS

Drugs	Time of Paralysis, Minutes
Cocain hydrochlorid, ½ per cent.	5×10
Novocain hydrochlorid, ½ per cent.	10×15
Cocain hydrochlorid, ¼ per cent. + novocain hydrochlorid, ¼ per cent.	5×10
Cocain hydrochlorid, ¼ per cent.	20×30
Novocain hydrochlorid, ¼ per cent	20×40
Cocain and novocain hydrochlorids, of each ⅛ per cent. ...	80×95
Cocain hydrochlorid, ½ per cent.	5×10
Quinin urea hydrochlorid, 2 per cent.	20×30
Cocain hydrochlorid, ¼ per cent. + quinin urea hydrochlorid, 1 per cent.	20×30

VI. MIXTURES OF THE ANESTHETICS AND POTASSIUM

This problem was worked out as in the first paper. The "M. E. C." (minimal effective concentration) refers to complete block within thirty minutes. The detailed results, presented in Table 6, show that there is no potentiation; indeed, scarcely even simple summation.

This is in marked contrast to the effects on motor-fibers, in which the efficiency was potentiated to eight times. I have not at this time any explanation to offer for the difference.

TABLE 6.—MIXTURES OF POTASSIUM AND ANESTHETICS ON SCIATIC SENSORY FIBERS OF FROG

Ratio of M. E. C. of Synergist : KCl = Total	Per Cent. of Cocain, Novocain, Tropacocain or Alypin hydrochlorids (M. E. C. = 0.25 per Cent.)	Per Cent. of KCl (M. E. C. = 0.5 per Cent.)	Time of Paralysis in Mixtures					
			Cocain KCl	Novocain KCl	Tropacocain KCl	Alypin KCl	Quinin Urea KCl	Per Cent. of Quinin Urea hydrochlorid (M. E. C. = 1 per Cent.)
1:0 = 2	1/2	0	5×10	10×15	5×10	......	20×30	2.0
1:1 = 2	1/4	1/2	5×10	5×10	5×10	......	5×10	1.0
0:1 = 2	0	1	5×10	5×10	5×10	......	5×10	0
2:1 = 1½	1/4	1/4	5×10	10×20	5×10			
1:0 = 1	1/4	0	20×30	20×40	20×40	......	>60	1.0
3:1 = 1	3/16	1/8	20×30	>60	30×50	......	>60	0.75
1:1 = 1	1/8	1/4	>60	>60	50×60	......	>60	0.5
1:3 = 1	1/16	3/8	20×30	>60	20×30	......	>60	0.25
0:1 = 1	0	1/2	20×40	20×40	20×40	......	20×40	0
2:1 = 3/4	1/8	1/8	50×65	30×50	10×20	50×65	......	0.5
1:0 = 1/2	1/8	0	30×50	>50	40×80	......	......	0.5
0:1 = 1/2	0	1/4	>50	>50	>50	......	......	0

CONCLUSIONS

1. The activity on sensory nerve fibers was studied by applying the anesthetics to the sciatic plexus and observing the presence or absence of reflex response to stimulation of the foot.

2. Cocain, novocain and tropacocain hydrochlorids are about equally efficient. The efficiency of potassium, alypin, quinin, urea, and especially antipyrin, is smaller; and their efficiency ratio in comparison with cocain is lower than for motor fibers.

3. Alkalization increases the efficiency of the organic anesthetic from two to eight times; the increase being generally about one-half as much as for motor fibers.

4. Epinephrin does not increase the efficiency; this result being the same as with the motor fibers.

5. Mixtures of cocain with novocain hydrochlorid or with quinin urea hydrochlorid give simple summation without potentiation, just as with the motor fibers.

6. Mixtures of the anesthetics with potassium do not show potentiation, differing in this respect from motor fibers.

Apparently, therefore, the sensory fibers show some important differences from motor fibers in their response to local anethetics.

COMPARISON OF ACTIVITY OF LOCAL ANESTHETICS

III. ANESTHESIA OF FROG-SKIN *

TORALD SOLLMANN
CLEVELAND

(From the Journal of Pharmacology and Experimental Therapeutics. February, 1918, p. 9.)

I. INTRODUCTION

Application of anesthetic solutions to the skin of frogs involves penetration, as well as the action on the finer sensory nerves. In view of the physiologic similarity of the frog-skin to mucous membranes, the method approaches clinical "surface anesthesia." It is simplified, however, since the removal of the drug by absorption is minimized through destruction of the circulation.

In practicing this method the anesthetic is usually applied to the skin of the frog's foot; the existence of anesthesia is ascertained by observing the presence or absence of reflex response to sensory stimulation of the foot.

This method has been used, rather incidentally, by Impens,[1] 1905; Issekutz,[2] 1912; Zorn,[3] 1913; Fromherz,[4] 1914. Their results were not very satisfactory. This is explainable, at least in part, by certain details of procedure, which vary in the different investigations, for example, failure to simplify the reflexes by decapitation, and the absorption by excision of the heart; the

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* From the Pharmacological Laboratory of the School of Medicine, Western Reserve University.

* This investigation is partly supported by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

[1] Impens: Deutsch Med. Wchnschr., 1905, No. 29; Arch. f. d. ges. Physiol., **110**: 21, 1905.

[2] Issekutz: Arch. ges. Physiol., **145**: 448, 1912.

[3] Zorn: Ztschr. f. exper. Path. u. Therap., **12**: 529, 1913.

[4] Fromherz: Arch. f. exper. Path. u. Pharmakol., **76**: 257, 1914.

use of delayed reaction, rather than absence of reaction, as criterion of paralysis; too short a time of immersion in the anesthetic solution, thus exaggerating the factor of rapidity of penetration. It does not seem necessary to discuss these papers further. The following simple technic gave very satisfactory results in my hands:

Method.—The frogs were decapitated, leaving the lower jaw. The heart was excised. The frogs were suspended from the jaw. Each leg was immersed in a homeopathic vial containing 6 to 8 c.c. of the anesthetic solution, reaching about midway to the knees. A cuff of adhesive plaster about both thighs prevented the frog from removing its legs from the solution.

At the end of every ten minutes the legs were withdrawn and the foot immersed in tenth-normal HCl. If the animal reacted by sharply jerking the foot from the acid, the leg was washed in water and replaced in the solution; this procedure being repeated every ten minutes during an hour, or until the paralysis was completed. Intactness of the reflex centers could be confirmed by applying the acid to the leg above the level of the immersion.

II. RELATIVE EFFICIENCY OF THE ANESTHETICS ON FROG-SKIN

The mean results are shown in Table 1, and more conveniently in Table 2.

Table 2 brings out a rather striking phenomenon: With direct immersion of motor and sensory fibers (Table *2* of Papers 1 and *2*) the ratios are nearly equal for the concentrations that paralyze rapidly and those that paralyze slowly. With the frog's foot, on the other hand, there is a striking difference. This is evidently due to differences in rapidity of penetration. The cocain apparently penetrates rapidly, and therefore has a very high ratio at first; the others penetrate more slowly, and thus their relative efficiency rises as time progresses. After forty minutes, the results become unreliable, because these late reactions depend so much on the survival period of the nervous centers.

TABLE 1.—TIME OF COMPLETE SENSORY PARALYSIS OF FROG-SKIN *

Per Cent. of Drug	Cocain Hydrochlorid	Novocain Hydrochlorid	Tropacocain Hydrochlorid	Alypin Hydrochlorid	KCl	Quinin Urea Hydrochlorid	Antipyrin (in water)	$NaHCO_3$
10	......	......	......	......	......	......	×10	
8	×10							
4	×10							
2	×10	......	×10	......	×10	20×30	×10	
1	×10	×10	×10	×10	10×20	30×40	20×30	
½	×10	10×20	......	10×20	20×30	40×50	30×40	50×60
¼	×10	40×60	×10	×10	50×60			
⅛	40×50	40×50	10×20	×10				
1/16	......	30×40	20×30	20×40				
1/32	......	......	10×60					
1/64	......	......	20×40					

* In this and all other tables, the numbers to the left of the × denote the time (minutes) when there was no paralysis; and the numbers to the right of the × denote the time (minutes) when paralysis was completed.

TABLE 2.—CONCENTRATIONS PRODUCING SENSORY PARALYSIS OF FROG'S FOOT IN A GIVEN TIME

	Time of Sensory Paralysis					Ratio of Efficiency (Paralytic Concentration in Terms of Cocain Hydrochlorid = 1)				
Minutes	0-10	10-20	20-30	30-40	>40	0-10	10-20	20-30	30-40	>40
Drugs	Percentage					Ratio				
Cocain hydorchlorid	⅛	..	..	..	1/16	1	1	1	1	1
Novocain hydrochlorid	1	½	..	..	¼	⅛	⅕	..	..	¼
Tropacocain hychlorid	¼	⅛	1/16	1/32 and 1/64	..	½	⅘	1⅓	2 to 5	
Alypin hydrochlorid	⅛	..	..	1/16	..	1	..	..	1	
KCl	4	2	1	..	½	1/32	1/20	1/12	..	⅛
Quinin urea hydrochlorid	..	..	4	2	1	..	..	⅛	1/21	1/16
Antipyrin (in water)	4	..	2	1	..	1/32	..	½	1/14	

TABLE 3.—EFFICIENCY RATIOS

			Frog Skin		
	Motor Fibers	Sensory Fibers	Within 10 Minutes	20 to 30 Minutes	After 40 Minutes
Cocain chlorid	1	1	1	1	1
Novocain hydrochlorid	1	1	⅛	⅕	¼
Tropacocain hydrochlorid	1	1	½	⅓	5
Alpin hydrochlorid	¾	½	1	1	1
KCl	1	½	1/32	1/12	⅛
Quinin urea hydrochlorid	⅕	⅛	>1/50	1/50	1/16
Antipyrin	⅛	1/32	1/32	1/24	1/10

The approximate efficiency ratios are compared in Table 3.

Table 3 illustrates that the efficiency ratios for conduction anesthesia and for surface anesthesia are quite unrelated. Potassium chlorid, for example, is an excellent agent for conduction of anesthesia, but practically useless for prompt surface anesthesia. Novocain shows up rather poorly, whereas alypin and especially tropacocain appear favorably.

The absolute concentrations required to produce anesthesia are compared with those for immersion of the motor nerve, in Table 4.

III. EFFECTS OF ALKALI

In these determinations sodium bicarbonate, generally ¼ per cent., was added to the anesthetic. The mean results are shown in Table 5, and compared with direct immersion in Table 6.

It is seen that alkalization increases the efficiency very materially, even more in the case of the frog-skin than with surface anesthesia. This indicates that the free bases are better able to penetrate the skin, as well as the nerve fibers.

IV. EFFECT OF EPINEPHRIN

The addition of epinephrin (1:20,000) to cocain hydrochlorid ⅛ per cent. and novocain ¼ and ⅛ per cent., does not alter the time of paralysis. It may be recalled that the results with direct immersion were also negative.

V. MIXTURES OF COCAIN WITH NOVOCAIN AND QUININ UREA HYDROCHLORIDS

The frog-skin gave rather imperfect summation, and did not give potentiation (Table 7). This agrees with direct immersion.

VI. MIXTURES OF THE ANESTHETICS WITH POTASSIUM

This problem was again worked out as in the first paper. The detailed results, as presented in Table 8, show no potentiation with cocain and novocain; agreeing with direct immersion of the sensory fibers, but in contrast to the motor fibers.

TABLE 4. COMPARISON OF PERCENTAGE OF THE ANESTHETICS REQUIRED TO ANESTHETIZE THE MOTOR NERVES AND THE FROG'S SKIN

	Motor Nerve (Immersion from 30 to 45 Minutes)	Frog-Skin (Immersion from 20 to 30 Minutes)
Cocain hydrochlorid	1/8	1/8
Novocain hydrochlorid	1/8	1/2
Tropacocain hydrochlorid	1/8	1/16
Alypin hydrochlorid	1/8–1/4	1/16
KCl	1/4	1
Quinin urea hydrochlorid	1	4
Antipyrin	1	2

TABLE 5. EFFECTS OF ALKALI ON FROG'S SKIN. COMPARISON OF THE MEAN TIME OF PARALYSIS, WITH THE HYDROCHLORIDS AND BASES (ADDITION OF ¼ PER CENT. OF SODIUM BICARBONATE)

	Cocain		Novocain		Tropacocain		Alypin			
Per Cent.	Hydrochlorid	Base	Hydrochlorid	Base	Hydrochlorid	Base	Hydrochlorid	Base	KCl	KCl + $NaHCO_3$ (1/2%)
4	....	....	.....	......	......	......	......	..	×10	×10
2	....	....	.....	......	......	......	......	..	10×20	20×30
1	....	....	×10	......	......	......	......	..	20×30	
1/2	....	....	10×20							
1/4	...	....	40×60	10×20	×10					
1/8	×10	....	......	×10	102×0	......	×10			
1/16	40×50	....	......	20×40	20×30	×10	20×40	×10		
1/32	......	....	......	20×30	10×60	20×30				
1/64	......	×10	......	20×30	20×40	40×50				
1/128	......	10×20								
1/256	......	30×40								
Efficiency ratio	8	16	4	8	2	4				

TABLE 6.—INCREASE OF EFFICIENCY SECURED BY ALKALIZATION

	Motor Fibers, Times	Sensory Fibers, Times	Frog Skin, Times
Cocain	8	2–4	8–16
Novocain	8	2–4	4–8
Tropacocain	2–8 (mean 4)	2–4	2–4
Alypin	4–8 (mean 6)	2–8	

TABLE 7.—MIXTURES OF COCAIN WITH NOVOCAIN AND QUININ UREA HYDROCHLORIDS

Drugs	Time of Paralysis, Minutes
Cocain, ⅛ per cent.	×10
Novocain, ½ per cent.	10×20
Cocain, 1/16 per cent. + novocain, ¼ per cent.	20×30
Quinin urea, 4 per cent.	20×30
Cocain, 1/16 per cent. + quinin urea, 2 per cent.	30×40

TABLE 8.—MIXTURES OF POTASSIUM WITH COCAIN AND NOVOCAIN HYDROCHLORIDS ON FROG-SKIN

Ratio of M. E. C.* Synergist: KCl = Total	Per Cent. of KCl (M. E. C. = 2 per Cent.)	Per Cent. of Cocain Hydrochlorid (M. E. C. = ⅛ per Cent.)	Cocain KCl, Time	Per Cent. of Novocain Hydrochlorid (M. E. C. = ½ per Cent.)	Novocain KCl, Time
1:0 = 1	0	⅛	×10	½	10×20
3:1 = 1	½	3/32	20×40	⅜	20×40
1:1 = 1	1	1/16	50×60	¼	20×30
1:3 = 1	1½	1/32	40×50	⅛	20×30
0:1 = 1	2	0	10×20	0	10×20
1:0 = ½	0	1/16	40×50	¼	40×60
3:1 = ½	¼	3/64	......	3/16	10×20
1:1 = ½	½	1/32	......	⅛	30×40
1:3 = ½	¾	1/64	......	1/16	40×50
0:1 = ½	1	0	20×30	0	20×30
1:0 = ¼	0	1/32	50×	⅛	40×50
3:1 = ¼	⅛	3/128	......	3/32	40×50
1:1 = ¼	¼	1/64	......	1/16	50×60
0:1 = ¼	½	0	50×60	0	50×60

* M. E. C. = minimal efficient concentration producing paralysis inside of twenty minutes.

CONCLUSIONS

1. The application of the anesthetics to the skin of the frog's foot approaches the conditions of surface anesthesia, involving penetration of the drug as well as action on the sensory fibers; but somewhat simplified by doing away with the absorptive removal of the drug. The results differ in some important respects from those of direct immersion (conduction anesthesia).

2. The efficiency ratios for the frog-skin are different for prompt and for slow anesthesia. Cocain, alypin and tropacocain make the best showing. Novocain is relatively less efficient than for conduction anesthesia;

potassium, quinin urea and antipyrin make a very poor showing for fast anesthesia; much better for slow anesthesia.

3. Alkali increases the efficiency, even more than for immersion.

4. Epinephrin does not increase the efficiency.

5 and 6. Mixtures of cocain with novocain or quinin do not potentiate; nor do mixtures of potassium with cocain or novocain.

COMPARISON OF ACTIVITY OF LOCAL ANESTHETICS

IV. ANESTHESIA OF RABBIT'S CORNEA [*]

TORALD SOLLMANN
CLEVELAND

(From the Journal of Pharmacology and Experimental Therapeutics, February, 1918, p. 17.)

I. INTRODUCTION

The application of anesthetics to the eye is a typical instance of "surface anesthesia." It involves, not only the action on the nervous elements, but also the factors of penetration through the mucous membrane; and of the disappearance of the drugs by further absorption. The conjunctival "sac" is exceptionally well arranged for keeping the solution in contact with the mucosa. The winking reflex furnishes a simple objective criterion of sensation. The method is also attractive because its results can be transferred directly to clinical practice. It has, accordingly, been used rather extensively; sometimes on the human eye (Schlueter,[1] 1907), sometimes on animals, especially rabbits (Impens,[2] 1905; Morgenroth and Ginsberg,[3] 1912, 1913; Closson,[4] 1914; Fromherz,[5] 1914.

The human eye is especially valuable for differentiating various grades of irritation. It is not quite safe, however, if fairly high concentrations are to be tested.

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[*] From the Pharmacological Laboratory of the School of Medicine, Western Reserve University.

[*] This investigation is partly supported by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.,

[1] Schlueter, H.: Klin. Monastbl. f. Augenh., **14:** (II), 198, 1907.

[2] Impens: Deutsch. med. Wchnschr., 1905, No. 29; Arch. f. d. ges. Physiol., **110:** 21, 1905.

[3] Morgenroth and Ginsberg: Berl. klin. Wchnschr., **49:** 2183, 1912; ibid., 1913, **50:** 343.

[4] Closson: J. Mich. State Med. Soc., **13:** 587, 1914.

[5] Fromherz: Arch. exper. Path. u. Pharmakol., **76:** 257, 1914.

Further, the comparative tests would need to be made on a single individual, to discount subjective variations. This draws out the work to undesirable lengths of time.

The sluggish temperament of rabbits largely does away with these disturbing factors; so that the method, as applied in this work, gives remarkably uniform results. This does not appear to have been the universal experience; for individual differences in the animals are often mentioned by other investigators. In some cases this is explainable by the short time of application, which emphasizes unduly accidental differences in the rate of absorption. In other cases, the method of testing the anesthesia such as "deep pressure" or electric stimulation, also places more emphasis on the depth of penetration than on the depth of anesthesia. Because of these differences the results are not directly comparable.

Method.—As in all experiments on local anesthesia, comparative experiments must be made by a rather rigidly detailed technic. The following steps (based partly on Morgenroth and Ginsburg) were followed: The rabbits were confined in snugly fitting, but comfortable, individual stalls, leaving the head free. The lashes around the eyelids were clipped. The winking reflex was confirmed by touching the cornea with a pointed lead pencil. The lower eyelid was pinched into a pocket, into which was inserted the point of an eye-dropper, filled with the anesthetic solution. The conjunctival sac was thus kept flooded for one minute, when the pipette was withdrawn and the rabbit released. In ten minutes the winking reflex was tested by touching the cornea, near the center, with the pencil point. If this did not produce winking, the pencil was drawn across the cornea several times with moderate pressure. Great care must be taken to avoid touching the eyelid or the hairs about the eye.

If the winking occurred with the first light touch the result was considered negative as to anesthesia. If the winking occurred, but only sluggishly and after repeated and fairly severe pressure, it was interpreted as partial anesthesia. Absence of winking constituted the sign of complete anesthesia.

If after the end of the first period the anesthesia was absent or only partial, the conjunctival sac was again filled with the solution; but the rabbit was not held for a minute, being released at once.

The tests and applications were repeated every ten minutes until four applications had been made; unless complete anesthesia had been obtained before. No applications were made after anesthesia was complete.

The tests, however, were continued for fifty minutes or longer, to observe the time of partial and complete recovery (partial anesthesia and normal sensitiveness).

The solutions, as usual, were made with eighth-molecular NaCl (0.73 per cent). Holocain and beta eucain were added to the list, since these are rather extensively used in eye work.

II. RELATIVE EFFICIENCY OF THE ANESTHETICS ON THE CORNEA

The condensed mean data are shown in Table 1. The table has been condensed so as to show only the mean data of the most significant factors, namely, complete anesthesia and complete recovery. The details of partial anesthesia and partial recovery have been omitted.

TABLE 1.—ANESTHETICS ON RABBIT'S CORNEA

Percentage	Cocain Hydrochlorid		Novocain Hydrochlorid		Tropacocain Hydrochlorid		Alypin Hydrochlorid		Holocain Hydrochlorid		Beta Eucain Hydrochlorid		KCl	Quinin Urea Hydrochlorid		Antipyrin	$NaHCO_3$
	A*	R	A	R	A	R	A	R	A	R	A	R	A	A	R	A	A
10.0	..	..	..	.	..	..	..	..	..	..	..	..	None	..	..	None	
8.0	..	..	2	50	2	30											
4.0	..	..	Incomplete		1	40	..	..	..	..	1	50	......	..	..	None	
2.0	2	30	None		1	15	2	30	..	..	3	20	None	1	>65	....	None
1.0	2	20	....		None		Uncertain		3	30	2	30	....	Incomplete			
0.5	2	10	None		None		Uncertain		2	20	None		....	None			
0.25	None		....		....		None		Incomplete								
0.125	None																

* See the text for explanation of the symbols.

In all these tables "A" signifies the number of applications (at ten minutes intervals) required to produce complete anesthesia; "R" signifies the minutes elapsing between the last application and complete recovery of sensation. "Incomplete" signifies that the cornea was evidently less sensitive, but still reacted. "None" signifies no apparent anesthesia. "Irregular" signifies that the results were confused, about an equal number of experiments giving positive and negative results.

Table 2, "A" shows the minimal concentrations giving complete anesthesia; and compares these with the concentrations required for motor nerves and for frog-skin; "B" shows the efficiency ratios in terms of cocain hydrochlorid = 1.

TABLE 2.—MINIMAL EFFECTIVE CONCENTRATIONS AND EFFICIENCY RATIOS

	A. Concentrations (per Cent.)			B. Efficiency Ratios (Cocain Hydrochlorid = 1)			
	Rabbit's cornea	Frog Skin (immersion 20 to 30 minutes)	Frog motor nerve (immersion 30 to 45 minutes)	Rabbit's cornea	Frog Skin (immersion 20 to 30 minutes)	Sensory Fibers	Motor Fibers
Cocain hydrochlorid.......	½	1/8 to 1/12	⅛	1	1	1	1
Novocain hydrochlorid....	8.0	½	⅛	1/16	⅕	1	1
Tropacocain hydrochlorid	2.0	1/16	⅛	¼	1⅓	1	1
Alypin hydrochlorid.......	2.0	1/16	⅛ to ¼	¼	1	½	¾
Holocain hydrochlorid....	½	....	....	1			
Beta eucain hydrochlorid..	1.0	....	....	½			
KCl........................	>10.0	1.0	¼	<1/20	1/12	½	1
Quinin urea hydrochlorid..	2.0	4.0	1.0	¼	1/50	⅛	⅕
Antipyrin.................	>10.0	2.0	1.0	<1/20	1/24	1/32	⅛

The tables illustrate again the danger of transferring results without judgment; for example, novocain, one of the best anesthetics when brought into direct contact with the nerves, is very weak when applied to mucous membranes. The efficiency of cocain on mucosae is superior to all its competitors. Beta eucain and holocain show up well; tropacocain and alypin fairly well. Quinin-urea makes a surprisingly good showing. Potassium and antipyrin appear useless.

Table 1 also permits some conclusions as to the duration of action. This depends largely on the strength of the solution. With cocain, for example, complete recovery required thirty minutes after the application of 2 per cent.; twenty minutes after 1 per cent. and ten minutes after 0.5 per cent. Comparisons may be made with the just effective concentrations. According to the time required for complete recovery, the anesthetics may be divided into groups, as in Table 3.

TABLE 3. RECOVERY FROM LOCAL ANESTHETICS

Complete recovery occurs in x minutes after last application of minimum effective concentrations of the hydrochlorid to the corneo	
10 to 15 minutes	Cocain, tropacocain
20 to 30 minutes	Alypin, beta eucain, holocain
50 minutes	Novocain
65 minutes	Quinin urea

The rapidity of action is probably also influenced by the concentration, although this is not apparent in the experiments. Groups may be distinguished as in Table 4.

TABLE 4.—RAPIDITY OF ACTION OF LOCAL ANESTHETICS (HYDROCHLORIDS) ON THE CORNEA

Mean number of applications at ten minute intervals required to produce complete anesthesia	
One application	Tropacocain (1 to 4 per cent.) Alypin (½ to 2 per cent.) Quinin urea (2 per cent.)
One to two applications	Beta eucain (1 to 4 per cent.)
Two applications	Cocain (½ to 2 per cent.) Novocain (8 per cent.) Holocain (½ to 1 per cent.)

TABLE 5.—EFFECTS OF ALKALIZATION ON CORNEAL ANESTHESIA

Percentage	Cocain		Novocain		Tropacocain		Alypin		Holocain		Beta Eucain		Quinin Urea	
	A*	R*	A	R	A	R	A	R	A	R	A	R	A	R
3.0	..	..	..	..	1	40								
2.0	..	..	Uncertain		1	20	..	..	..	..	..	..	None	
1.0	..	..	Incomplete		1	30	..	..	..	..	..	..	None	
½	1	40	Incomplete		1	15	4	40	..	..	1	10	4	>20
¼	Uncertain				None		Incomplete		Incomplete (precipitations)		Incomplete		Incomplete	
⅛	None						Incomplete		Incomplete (precipitations)		Incomplete			
1/16	None													

* A = number of applications required for complete anesthesia. R = minutes between last application and complete recovery.

Sodium bicarbonate, 0.25 per cent., was added to the anesthetics. This table should be compared with Table 1. The abbreviations have the same significance. Only the mean data are given.

III. EFFECTS OF ALKALI

These were determined by adding ¼ per cent of sodium bicarbonate to the anesthetic. It was first shown that a 1 per cent solution of sodium bicarbonate is not anesthetic or irritant.

The condensed data are given in Table 5.

TABLE 6.—RESULTS OF ALKALIZATION

	Rabbit's Cornea: Minimal Effective Percentage of the		Increase of Efficiency Secured by Alkalization			
Anesthetic	Hydrochlorid	Base (bicarbonate)	Cornea, Times	Frog Skin, Times	Sensory Fibers, Times	Motor Fibers, Times
Cocain............	½	¼ to ½	1 to 2	8 to 16	2 to 4	8
Novocain..........	8.0	2 to 4	2 to 4	4 to 8	2 to 4	8
Tropacocain.......	2.0	½	4	2 to 4	2 to 4	2 to 8
Alypin............	2.0	½	4	......	2 to 8	4 to 8
Holocain..........	½	>¼*				
Beta eucain.......	1.0	½	2			
Quinin urea........	2.0	>2.0	Less efficient			

* Holocain, ¼ per cent. and even ⅛ per cent., is precipitated by bicarbonate.

The minimal efficient concentration and the efficiency ratio are compared in Table 6. The ratios for the skin and for the immersed nerves are given for comparison.

It is seen that alkalization increases the efficiency of the typical anesthetics, acting on the cornea, up to four times—about as much as with the other methods of application. However, the ratio of increase cannot be transferred from one method to the other. For instance, cocain is most powerfully potentiated by alkali in the case of the frog-skin; and very little for the cornea. This suggests that the quantitative effects of alkalization must probably be worked out for each clinical use. The principle of its benefits, however, stands secure. The rapidity and duration of the anesthesia does not appear to be influenced in any definite manner.

Quinin-urea differs from the other anesthetics. It was rendered distinctly less effective by alkalization. No precipitate was observed.

IV. EFFICIENCY OF EPINEPHRIN

The addition of epinephrin, 1 : 20,000, to cocain hydrochlorid, ½ and ¼ per cent.; and to quinin-urea hydrochlorid 2 per cent, rather diminished their efficiency. It is therefore practically inadvisable. This agrees with the results of the other methods so far as studied.

V. MIXTURES OF COCAIN WITH ALYPIN AND WITH QUININ-UREA HYDROCHLORIDS

The experiments shown in Table 7 demonstrate that there is no potentiation. On the contrary, the summation appears to be imperfect.

TABLE 7.—MIXTURES OF ANESTHETICS APPLIED TO THE CORNEA

Cocain hydrochlorid, ½ per cent..	Complete anesthesia after two applications
Alypin hydrochlorid, ½ per cent...	Uncertain
Mixtures of equal volumes of both solutions....................	No anesthesia
Quinin urea hydrochlorid, 2 per cent.	Complete anesthesia after one application
Mixture of equal volumes of 2 per cent. quinin urea and ½ per cent. cocain solution....................	No anesthesia

VI. MIXTURE OF THE ANESTHETICS WITH POTASSIUM

Mixtures of the anesthetics with various proportions of 10 per cent. KCl gave about the same results as if they had been diluted with plain NaCl. There was certainly no potentiation. The following anesthetics (hydrochlorid) were tried: Cocain, alypin, holocain, each ½ per cent; quinin-urea, 2 per rent.

VII. COMPARISON OF EXPERIMENTAL AND CLINICAL DATA

The experimental results furnish remarkable confirmation of the clinical experiences; as recorded, for example, in Wood's "Ophthalmic Therapeutics." They explain why cocain is still considered the superior anesthetic for mucous membranes, and why novocain has failed to establish itself in this field. They explain also why the addition of epinephrin, and of potassium, has not become popular for surface anesthesia. Little or nothing is to be gained by combination of anesthetics, from the standpoint of efficiency, although they may minimize undesirable side-effects. The addition of sodium bicarbonate seems worthy of further clinical trial.

VIII. CONCLUSIONS

1. The application of anesthetics to the cornea is a satisfactory method for comparing their efficiency as surface anesthetics. Presumably the results would apply also to other mucous membranes, although this has not been tested directly.

2. The order of efficiency is markedly different from immersion or conduction anesthesia. On the cornea, cocain and holocain are the most efficient; then follow beta-eucain, alypin, quinin-urea and tropacocain; and lastly, novocain. Antipyrin and potassium are practically ineffective.

The rapidity and the duration of the action vary with the concentration. For just effective concentrations, the duration is shortest with cocain and tropacocain, and longest with quinin-urea.

3. The addition of sodium bicarbonate (¼ per cent.) increases the efficiency of the anesthetics considerably (two to four times); with the exception of quinin-urea, which is rendered less efficient.

4. Epinephrin does not increase the efficiency.

5-6. Mixtures of the anesthetics with each other or with potassium, do not lead to potentiation.

7. The experimental data are in agreement with the clinical usage.

COMPARATIVE EFFICIENCY OF LOCAL ANESTHETICS

V. ANESTHESIA OF THE HUMAN SKIN. GENERAL CONCLUSIONS *

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I. INTRODUCTION

The local anesthesia of the wheals produced by the intracutaneous injection of the anesthetics involves the finest nerve fibrils, and possibly the sensory endings. It therefore minimizes the complicating factor of penetration. This would render it very suitable for the comparison of the absolute anesthetic power. However, the drugs disappear fairly rapidly by systemic absorption. This may be avoided, either by confining the observations to short intervals; or by combining the drugs with epinephrin. The end-point—the absence of sensation—is somewhat subjective and not very delicate; but its accuracy is about that of the other methods and sufficient for practical purposes. The method is therefore the best for comparing absolute anesthetic efficiency. It has the added advantage that its results can be transferred directly to one of the clinical uses, namely superficial infiltration anesthesia; and it approaches quite closely the conditions of intraneural and conduction anesthesia. It cannot be applied to surface anesthesia.

Because of these advantages, the wheal or "quaddel" test is being used quite extensively since its introduction by H. Braun: by Braun and his pupils (Reeke and Heinze[1]); Heinecke and Laewen,[2] 1905; Laewen,[3] 1910; Schmid,[4] 1913; Gros,[5] 1910; Hoffmann and Kochmann,[6] 1912, 1914; Hoffmann,[7] 1914; Kochmann,[8] 1914.

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* From the Pharmacological Laboratory of the School of Medicine, Western Reserve University.

* This investigation was partly supported by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

Braun held that the anesthetic strength could be determined either by the duration of the anesthesia or by the minimal effective concentration. The duration is a rather misleading criterion; for the return of sensation depends upon the absorptive removal of the drug, and not on the intensity of the anesthetic effect. The duration of the anesthesia is important from a clinical standpoint; but the anesthetic efficiency must be judged by the minimal concentration that will produce a given anesthetic effect. Fortunately, most of the workers have adopted this as their criterion.

The absolute results of the various experimenters differ rather widely, as will be shown later. This may be due to differences in the subjects of the experiments. More probably, however, the results varied according to the method of ascertaining the "anesthesia." A careful and uniform technic is essential to comparable results.

Technic.—I followed essentially the technic of Hoffmann and Kochmann,[6] 1914: The flexor surface of the arm was scrubbed with alcohol. The injections were made into (not under) the skin, through a fine needle attached to a Luer syringe. It was aimed to produce a wheal of 7 to 10 mm., requiring 0.3 to 0.4 c.c. of solution; but the exact size and quantity are immaterial.

Hoffmann and Kochmann advise that all the wheals be made on one side, either the radial or ulnar. I prefer to make

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1. Braun, H.: Deutsch. Med. Wchnschr., 1905, p. 1668; Zentralbl. f. Chhir., **40:** 1513, 1913; Local Anesthesia (English translation by P. Shields, 1914).

2. Heinecke and Laewen: Deutsch. Ztschr. f. Chir., **80:** 180, 1905.

3. Laewen: München. med. Wchnschr., **57:** 2044, 1910.

4. Schmid, A.: Ztschr. f. Exper. Path., **14:** 527, 1913.

5. Gros, O.: Arch. f. Exper. Path. u. Pharmakol., **62:** 380, 1910; ibid., **63:** 80.

6. Hoffmann and Kochmann: Deutsch. med. Wchnschr., **48:** 2264, 1912; Beitr. f. klin. Chir., **91:** 489, 1914..

7. Hoffmann, A.: Deutsch. med. Wchnschr., **39:** 1798, 1914

8. Kochmann, M.: Therap. Monatsch., **28:** 646, 1914.

three wheals across the arm, and demand that at least two of these must show anesthesia.

The solutions, as usual, were made with eighth-molecular NaCl (0.73 per cent.). The solutions were made in series of 1, ½, ¼, ⅛, etc.

Hoffmann and Kochmann,[6] 1914, tested the anesthesia by a bit of cotton twisted to a fine point. I also found this the most satisfactory, because the most objective. At first, I used a fine copper wire with its end fused into a ball. This was applied by stroking and pressure. It showed complete anesthesia with exactly the same concentrations as the cotton; but it also showed disturbed sensation with more dilute solutions. This proved to be confusing rather than helpful, so that the later tests were made exclusively by the cotton method.

All the experiments were made on the author, except that additional experiments on the combined action of potassium and novocain were made on two students.

TABLE 1.—MINIMAL ANESTHETIC CONCENTRATIONS FOR HUMAN SKIN

Drug	Sollmann			Hoffmann and Kochmann			Reeke		Heinze	
	Per Cent. of Drug	Duration of Anesthesia, Minutes	Efficiency Ratio cocain = 1	Per Cent. of Drug	Duration of Anesthesia, Minutes	Efficiency Ratio cocain = 1	Per Cent. of Drug	Efficiency Ratio cocain = 1	Per Cent. of Drug	Efficiency Ratio cocain = 1
Cocain hydrochlorid.......	1/32	10	1	1/80	7	1	1/100	1	1/200	1
Novocain hydrochlorid....	1/32	2	1	1/40	6	½				
Tropacocain hydrochlorid	1/32	2	1	1/80	5	1	1/100	1	1/800	2
Alypin hydrochlorid.......	1/32	5	1	1/10	11	⅛				
Beta eucain hydrochlorid..	1/16	2	½	1/40†	7	½	1/100	1	1/200	2
Apothesin* hydrochlorid..	¼	5	⅛							
Holocain hydrochlorid....	..	..	..	....	..	..	1/200	2		
Acoin....................	..	..	..	....	..	..	1/200	2	1/2000	1/10
Nirvanin..................	..	..	..	....	..	..	1/40	⅖		
Subcutin..................	..	..	..	....	..	..	¼	1/25		
Quinin urea hydrochlorid..	⅛	5	¼							
Antipyrin.................	¼	5	⅛							
Potassium chlorid.........	¼	2	⅛							
Potassium sulphate.......	¼	2 & 4 4	⅛	¼	7	1/20				

* Apothesin is a new proprietary anesthetic said to be the cinnamic ester of gamma-diethylamino propyl alcohol hydrochlorid. It was obtained through the kindness of Dr. McCoy, United States Public Healtth Service.

† Hoffmann and Kochmann used beta eucain lactate.

II. RELATIVE EFFICIENCY OF THE ANESTHETICS

Table 1 shows the minimal concentrations that render the wheal insensitive to the cotton, the duration of the anesthesia and the efficiency ratio in terms of cocain = 1.

Concentrations of one-half of those noted in the table did not produce complete insensibility to the twisted cotton.

In estimating the duration of anesthesia, the first test was made in two minutes, the succeeding tests every five minutes after injection (that is, duration of "five minutes" means that sensation was absent at the end of five minutes, but has returned by ten minutes).

The table also gives the data of other investigators who have used the wheal method for comparing the efficiency of anesthetics.

The table shows variations in the absolute concentrations, and even in the efficiency ratios; but these are no more than would be expected. They justify the following conclusions for the hydrochlorids:

Cocain, novocain, tropacocain and alypin act with about equal intensity, but alypin and especially cocain are more lasting than novocain and tropacocain. Beta-eucain anesthesia is also of short duration, and requires about double the concentration.

Quinin-urea requires four times the concentration of cocain.

Apothesin, antipyrin and potassium chlorid or sulphate require four times the concentration.

In Table 2, "A" compares the minimal concentrations giving complete anesthesia of the skin, with those for the cornea and nerves; "B" shows the efficiency ratios.

This table illustrates some interesting points: The actual concentration required to produce insensibility is lowest with the intracutaneous method—obviously because the factor of penetration is avoided, and all the nerve fibers in the field are exposed to the full concentration of the anesthetic. The cornea is at the other extreme, requiring the highest concentration, in order that the penetration through the epithelium may hold the balance to the absorptive removal.

The complicating factors of penetration and absorptive removal tend to divide the local anesthetics into two classes:

1. Those in which these factors play a small part, and which may therefore be used on mucous membranes. These may be called "surface-anesthetics."

2. Those in which these factors play a large part, and which can be used with advantage, only by injection (infiltration, intraneural, etc.). These may be called "injection anesthetics."

TABLE 2.—MINIMAL EFFECTIVE CONCENTRATIONS AND EFFICIENCY RATIOS

Drug	A. Concentrations (per Cent.)				B. Efficiency Ratios (Cocain = 1)				
	Intracutaneous	Frog motor Fibers	Frog skin	Rabbit cornea	Intracutaneous	Frog sensory Fibers	Frog motor Fibers	Frog skin	Rabbit cornea
Cocain hydrochlorid	1/32	⅛	⅛-1/12	½	1	1	1	1	1
Novocain hydrochlorid	1/32	⅛	½	8	1	1	1	⅕	1/16
Tropacocain hydrochlorid	1/32	⅛	1/16	2	1	1	1	1⅓	¼
Alypin hydrochlorid	1/32	⅛-¼	1/16	2	1	½	¾	1	¼
Beta eucain hydrochlorid	1/16	..	..	1	½	..	..	..	½
Holocain hydrochlorid	..	..	..	½	..	..	..	..	1
Apothesin hydrochlorid	¼	..	..	4*	⅛	..	..	..	⅛
Antipyrin	¼	1	2	>10	⅛	1/32	⅛	1/24	<1/20
Quinin urea hydrochlorid	⅛	1	4	2	¼	⅛	⅕	1/50	¼
Potassium chlorid	¼	¼	1	>10	⅛	½	1	1/12	<1/20

* Apothesin on rabbits' cornea: These experiments were made after Paper IV had been completed. They gave the following results:

8 per cent. solution: anesthesia on first application; persists between 10 and 30 minutes.

4 per cent. solution: anesthesia after second application; persists between 10 and 20 minutes.

2 per cent. and lower: no anesthesia, even after four applications.

A study of the table shows that the tests best adapted for emphasizing these two properties are: The cornea for surface-anesthesia; the intracutaneous method for injection-anesthesia. The other methods give intermediate and confirmatory results.

Table 3 shows the order of the anesthetics for these purposes, from the standpoint of absolute efficiency.

It must not be forgotten, of course, that anesthetic efficiency is not the only factor in the clinical application. Local irritation and systemic toxicity may be equally or more important, although they are not covered in this series.

TABLE 3.—ANESTHETIC EFFICIENCY

A. Injection Anesthesia		B. Surface Anesthesia	
		Cocain hydrochlorid	1
Cocain hydrochlorid	1	Holocain hydrochlorid	
Novocain hydrochlorid			
Tropacocain hydrochlorid		Beta eucain hydrochlorid	½
Alypin hydrochlorid			
		Tropacocain hydrochlorid	¼
Beta eucain hydrochlorid	½	Alypin hydrochlorid	
Quinin urea hydrochlorid	¼	Quinin urea hydrochlorid	
Apothesin	⅛	Apothesin	⅛
Antipyrin		Novocain	1/16
Potassium chlorid		Antipyrin	<1/20
		Potassium chlorid	

The duration of action would also enter into the "working efficiency." If the action is brief, then it is necessary to use a higher concentration in order to maintain the effective concentration to the end of the operation. However, with injection-anesthesia, the duration depends essentially on the absorptive removal; and since this can be modified at will, either by ligation or by epinephrin, the duration of action is not of great practical importance. Nevertheless, the data are worth recording (see Table 1).

With the minimal effective concentrations of the hydrochlorids, the duration is: cocain, ten minutes; alypin, apothesin, quinin-urea and antipyrin, five minutes; novocain, tropacocain, beta-uecain and potassium, two minutes.

The influence of the concentration on the duration is not as great as might be expected. The wire-method gave the following results:

Cocain, novocain, fropacocain and alypin hydrochlorids: duration of the anesthesia in 1/32 per cent. = five minutes; ⅛ per cent. and ½ per cent. = ten minutes.

Beta-ucain and quinin-urea hydrochlorids: ⅛ per cent. = five minutes; ½ per cent. = fifteen minutes.

Stronger concentrations have been tested by Braun,[1] 1903, 1905, 1914; Heinecke and Laewen,[2] 1905; Schmid,[4] 1913. The results of all are in substantial agreement, and may be condensed with those of Hoffmann and Kochmann,[6] 1914, and those of the author, into a single table (Table 4).

TABLE 4.—DURATION OF ANESTHESIA IN WHEALS. (COMPILED FROM THE AUTHORS CITED IN THE TEXT. THE DRUGS ARE USED AS HYDROCHLORIDS; THE TIME IS IN MINUTES)

Percentage	Cocain	Novocain	Tropacocain	Alypin	Beta Eucain	Quinin Urea	Stovain
10	.....	27	....	....	....	....	....
5	34-54	17-39	....	37	....	....	....
1	20-25	12-17	....	20	18	....	....
½	10-18	9-14	5-10	10	15-17	15	....
¼	8-14	5-10	....	....	....	....	....
⅛	6-15	½-10	5-7	¼-12	5-8	5	5-7
1/16	5-7	6½	....	....	½-5½	....	....
1/32	5	2-8½	5	5	3-10	....	....

The duration of the quinin anesthesia is a matter of practical interest. With dilute solutions, this is very little greater than that of the other anesthetics of equal efficiency. This suggests that the very much more enduring anesthesia ascribed to solutions of 1 per cent and stronger, is a different phenomenon than the ordinary anesthesia; consisting perhaps in a temporary injury of the nerve.

III. EFFECTS OF ALKALI

This was tested by adding ¼ per cent. of sodium bicarbonate to solutions of cocain and of novocain hydrochlorids. The results were disappointing; the alkaline solutions being distinctly less anesthetic than the same solutions without the alkali.

In the previous papers I have confirmed the favorable effects of alkalization on the penetration of the anesthetics; but this factor does not come into play in the intracutaneous method: the intimate contact with the nerve fibrils insures rapid penetration; and the normal alkaline reaction of the tissues would not be much influenced by the bicarbonate. The failure of the alkali is therefore easily explained. Gros (1910) and Laewen (1910) described favorable effects from alkali with the intracutaneous methods; but their results are not convincing.

The addition of bicarbonate would be useful in surface anesthesia, and for intraneural injection; but would be superfluous for infiltration.

IV. EFFICIENCY OF EPINEPHRIN

Braun introduced the anesthetic-epinephrin combination primarily for injection anesthesia, with the object of delaying the absorptive removal of the anesthetic. On this basis, the epinephrin would not influence the anesthesia if the circulation has been arrested; nor would it have much effect on the anesthesia of mucous membranes. This has been confirmed by the previous papers of this series. On the other hand, the present series confirms fully the claims of Braun: The epinephrin prolonged the injection-anesthesia very greatly —indeed, beyond the limits of the observations. The improvement concerned only the duration, not the intensity of the effect; that is, the maximal effective concentration was not altered. This confirms again that there is not synergism, in the ordinary sense.

The epinephrin was usually added to the concentration of 1:100,000. This produced intense blanching; at first there was also "gooseflesh." The blanching outlasted the effects of the anesthetics. Epinephrin by itself is not anesthetic.

Braun found that tropacocain differs from all the other anesthetics in being uninfluenced by epinephrin. This also was confirmed in the present series. Tropacocain alone, in concentrations as low as 1/32 per cent. produces an urticarial wheal and itching. When 1/8 per cent. tropacocain is injected with the epinephrin, it prevents the usual blanching for twenty-five minutes at the center of the wheal; but the periphery pales from the start.

The experiments are summarized in Table 5.

TABLE 5. INFLUENCE OF EPINEPHRIN ON INJECTION OF ANESTHESIA

Drug	Per Cent.	Duration of Anesthesia	
		With Epinephrin, Minutes	Without Epinephrin, Minutes
Cocain hydrochlorid	1/8	[illegible]	10×15
	1/32	>40	5×15
	1/64	0	0
Novocain hydrochlorid	1/8	60×105	[illegible]
	1/32	>30	5×10
	1/64	0	0
Tropacocain hydrochlorid	1/8	0×10	5×15
	1/32	5×10	5×10
Alypin hydrochlorid	1/8	>90	10×15
	1/32	[illegible]	5×10
Beta eucain hydrochlorid	1/8	[illegible]	5×15
	1/32	>26	0×10
Potassium sulphate	1/4	......	2×4
	1/8	5	0
	1/16	0	......
Quinin urea hydrochlorid	1/8	10×50	15×30
	1/32	0	0

* In this table the numbers to the left of the × denote the time (minutes) when there was no paralysis, and the number to the right of the × denotes the time (minutes) when paralysis was completed.

V. MIXTURES OF COCAIN, NOVOCAIN AND QUININ UREA

This was tested by mixing equal volumes of the just efficient concentrations of these anesthetics (cocain-novocain; cocain-quinin urea; quinin urea-novocain). These mixtures were, if anything, rather less anesthetic than the single drugs. When diluted with an equal volume of saline, they were ineffective.

The mixtures, therefore, give a somewhat imperfect summation, and not potentiation. This agrees with Schmid, 1913. It may be recalled that this was also the result with all of the other anesthetics.

VI. MIXTURES OF ANESTHETICS WITH POTASSIUM

Hoffmann and Kochmann[6] found considerable potentiation by mixing novocain and a potassium salt. This was confirmed in the first paper of this series, for the motor-fibers of frog's sciatic nerve. However, the sensory fibers and mucous membranes gave only imperfect summation.

Hoffmann and Kochmann,[6] 1912, further claim to have demonstrated a similar potentiation by the wheal method. They therefore suggested for clinical use a solution containing 0.4 per cent. of potassium sulphate with 0.05 to 0.25 per cent. of novocain. Braun, 1913, also claimed that there is some potentiation, but did not determine its degree; however, clinically he found the potentiation weak—so much so that he proposed to use the potentiation to "improve" rather than "replace" novocain. This would suggest summation rather than potentiation of action.

Hoffmann,[7] 1914, concedes that, clinically, 0.2 to 0.25 per cent. of novocain plus 0.46 per cent. of potassium are required to equal 0.5 per cent. of novocain. This appears to be little more than simple summation.

In 1914, Hoffmann and Kochmann,[6] and Kochmann,[8] published further detailed wheal experiments with potassium combinations. Mixing just anesthetic concentrations in equal parts and diluting them until ineffective, they record the following potentiations for combinations of the anesthetics with potassium sulphate:

Novocain	6
Cocain	4
Tropacocain	2
Beta eucain	1½
Alypin	½

The experiments that I made in this direction are shown in Table 6. They showed simple summation, but no potentiation. I have checked these results on two students. They all showed simple summation, and never any potentiation. I am at a loss to account for this difference from the results of Hoffmann and Kochmann; perhaps they may have been misled by using a single wheal, instead of three wheals, for each test. In any case, I must conclude that the potentiation, if it exists at all, is inconstant.

TABLE 6. MIXTURES OF POTASSIUM AND ANESTHETICS
Wheal Method; Cotton Test; Minimal Effective Concentrations and Duration of Anesthesia

Drug	Per Cent.	Duration of Anesthesia: Single Anesthetics, Minutes	Duration of Anesthesia: Plus an Equal Volume of ¼ per Cent. Potassium Sulphate, Minutes
Potassium sulphate	¼	2 to 4	..
Cocain hydrochlorid	1/32	10	5
Novocain hydrochlorid	1/32	2	2
Tropacocain hydrochlorid	1/32	2	2
Quinin urea hydrochlorid	⅛	5	2

All the solutions, single or mixed, were ineffective when diluted with an equal volume of saline solution.

CONCLUSIONS

1. The wheal method on the human subject gives probably the nearest approach to the absolute anesthetic power. Its results can be applied directly to injection anesthesia, but not to surface anesthesia. For the latter the corneal test is the most suitable.

2. The suitability of the different anesthetics for these two types of anesthesia are shown in Table 3. For injection anesthesia, cocain, novocain, tropacocain and alypin are about equally efficient; beta-eucain is one-half and quinin-urea is one-fourth as active; apothesin, antipyrin and potassium chlorid being one-eighth as active.

There are fairly large differences in the duration of action; but these differences are insignificant when compared with the differences that are produced by the addition of epinephrin.

3. The addition of sodium bicarbonate to cocain or novocain does not increase the activity. It would therefore not be useful, in contrast to surface or intraneural anesthesia.

4. The addition of epinephrin prolongs the action very greatly, except with tropacocain. The epinephrin does not, however, change the minimal efficient concentration.

5. Mixtures of cocain, novocain and quinin-urea hydrochlorids give somewhat deficient summation without potentiation. There would, therefore, be no advantage in such mixtures.

6. Mixtures of the anesthetics with potassium sulphate give only simple summation. This would be of some advantage in reducing the required amount of the anesthetic, the conditions being more favorable than with mucous membranes, which give not even summation; but too much should not be expected from the potassium mixtures.

THE COMPARATIVE ABSORBABILITY OF LOCAL ANESTHETICS FROM THE URETHRA *

TORALD SOLLMANN
CLEVELAND

(*From the Journal of Pharmacology and Experimental Therapeutics, March, 1918, p. 159.*)

INTRODUCTION

When local anesthetics are applied to mucous membranes, their efficiency and toxicity depend not only on their absolute anesthetic and toxic power, but also on their absorption. This varies undoubtedly for different drugs, and presumably also for different mucosae. The urethra is especially important, because of the many cases of poisoning arising from this surface. It is also technically convenient for the study of absorption. In principle, it is simply necessary to introduce a known quantity of the anesthetic into the urethra; to leave it there for a given period; to wash it out; and to estimate the unabsorbed aneshetic.

TECHNICAL DETAILS

The experiments were made on male dogs, anesthetized with morphin and ether. The urinary bladder was exposed through a suprapubic incision. An opening was made near the neck of the bladder. Through this an elbow cannula of small lumen and thick walls was introduced into the posterior urethra, and tied in position. The exterior end of the cannula was connected with a small stopcock, through which the injections were made. Another elbow cannula was tied into

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* From the Pharmacological Laboratory of the School of Medicine of Western Reserve University.

* This investigation was partly supported by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

the urethral meatus and used for the collection of the urethral fluid.

In making the observations, 50 c.c. of seventh-molecular sodium chlorid solution (0.73 per cent.) was forced through the urethra by means of an "atomizer bulb" connected with the pipet; the latter being connected with the stopcock in the bladder end of the urethra. The meatus cannula was then clamped, and the urethra was filled from the bladder end with 3, 4 or 5 c.c. of an anesthetic solution, according to the size of the dog. This required some pressure, but was nearly the same for all the solutions.

The stopcock was then closed, and the solution left in the urethra for twenty minutes (except in the experiments on the influence of time of sojourn). At the end of this period, the stopcocks were opened, and the urethra was rinsed with 50 c.c. of saline solution. Repeated tests with Mayer's reagent showed that this was more than sufficient to remove all of the unabsorbed anesthetic. The washings were collected in a bottle containing about 2 gm. of sodium chlorid—this to prevent emulsification in the extraction process.

The anesthetics were used in the form of the hydrochlorids. The 3 per cent. solutions used in most of the experiments contained 0.5 per cent. of NaCl. The 10 per cent. solutions were made without salt.

The quantitative estimation of the anesthetics was conducted by titrating the alkalinity of the bases, isolated by means of chloroform: The sample was divided into equal parts, for duplicate estimation. Each was rendered strongly alkaline with ammonia and shaken with successive portions of chloroform (at least five times), until the bases were completely extracted, as controlled by Mayer's reagent. The addition of about 5 per cent. of NaCl served to diminish emulsification, and thus hastened the process. Control experiments showed that it did not affect the accuracy of the determinations. The chloroformic solutions were filtered through cotton; most of the chloroform distilled off, and the residue then heated on a waterbath for about an hour, to assure the complete evaporation of any ammonia. The residue was then dissolved in approximately hundredth-normal sulphuric acid, and titrated with hundredth-normal sodium hydroxid, using methyl red as indicator.

The results actually obtained in control determinations were used for calculating the amount of the anesthetics, instead of the theoretical figures. Table 1 shows the theoretical equivalents, the percentages actually recovered and the conversion factors used in calculating the results.

TABLE 1.—ALKALIMETRIC METHOD OF ESTIMATION OF ANESTHETIC BASES. THESE CONTROL EXPERIMENTS WERE MADE WITH 1 C.C. OF 3 PER CENT. SOLUTION OF THE ANESTHETIC HYDROCHLORIDS

Anesthetic Hydrochlorid	1 C.c. N/100 Acid Should = Milligrams of Anesthetic Hydrochlorid	Per Cent. of Anesthetic Recovered in Control Experiments	Conversion Factor 1 C.c. N/100 Acid Is Accepted as Equivalent to Milligrams of Anesthetic Hydrochlorid
Cocain...................	3.40	94.6	3.57
Novocain*..............	2.73	89.0	3.05
Tropacocain............	2.66	94.0	2.84
Alypin...................	3.15	89.9	3.48
Beta eucain.............	3.02	92.2	3.27
Apothesin†.............	2.97 (?)	96.7 (?)	3.05

* Novocain: The specimen used in these absorption experiments was manufactured by the New York "Farbwerke-Hoechst Company."

† Apothesin is a new proprietary anesthetic, said to be the cinnamic ester of gamma-diethylamino propyl alcohol hydrochlorid. It was obtained through the kindness of Dr. McCoy, of the United States Public Health Service.

In the presentation of the results, the absorption is expressed as the percentage of the quantities introduced. These percentages are correct relatively, but not absolutely: Because of the dead space of the cannulae,[1] not all of the solution was in contact with the absorbing surface of the urethra. However, since the dead space was uniform in all of the experiments, the error is a constant one, and therefore does not interfere with the comparison of the solutions.

In making comparative experiments, it is of course desirable to have as many of the comparative observations as is possible on the same animal. With this object, from twelve to twenty solutions were used on each dog. This, however, introduces the possibility of other sources of error; namely, that the absorbing power of the urethra may be permanently altered by the presence of the successive solutions, or by the long etherization of the animal (the total duration of a series of experiments was about seven hours).

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[1] The dead space of the vesical cannula and its stop-cock and connections was about 1 c.c.

These sources of error were balanced by reversing the order of the administration of the drugs; so that a drug applied early in one series would be applied correspondingly late in another series.

TABLE 2.—ABSORPTION OF ANESTHETIC HYDROCHLORIDS FROM DOG'S URETHRA; 3 PER CENT. SOLUTION TWENTY MINUTES

Experiment number..	I	III 1st Series	III 2d Series	IV 1st Series	IV 2d Series	V
Quantity of the anesthetics given........	150 Mg.	150 Mg.	150 Mg.	120 Mg.	120 Mg.	90 Mg.
Order of administration*................	↓	↓	↑	↑	↓	↑
			A. Actual percentage absorbed			
Cocain................	76.0	31.5	42.7	31.4	31.4	70.6
Novocain.............	60.5	21.5	38.9	86.7	39.3	77.1
Tropacocain..........	85.5	37.9	39.1	55.0	45.3	55.6
Alypin................	78.9	35.3	45.0	75.8	60.1	46.0
Beta eucain...........	76.9	22.5	26.9	79.1	36.7	53.1
Apothesin............	85.3	24.6	22.1	89.9	39.7	41.1
Aver. of absorption of the six drugs....	77.2	29.0	35.8	69.7	42.1	57.8
			B. Percentage of the average absorption			
Cocain................	98.3	108.6	119.3	45.1	74.6	122.1
Novocain.............	78.3	74.1	108.7	124.4	93.3	133.2
Tropacocain..........	110.7	130.7	109.2	78.9	107.9	96.2
Alypin................	102.2	121.7	125.7	108.7	142.8	79.9
Beta eucain...........	99.6	77.6	75.4	113.5	87.2	92.0
Apothesin............	110.5	84.8	61.8	130.0	94.3	76.1

* ↓ — beginning with cocain and ending with apothesin. ↑ — beginning with apothesin and ending with cocain.

Different animals vary rather widely in their absorbing power. To permit their comparison, an "average of absorption" was established for each series of observations, by averaging the percentage of absorption of the six drugs used. The relative absorbability of each drug may then be expressed, as a percentage of this "average of absorption."

COMPARATIVE ABSORPTION OF THE ANESTHETICS

The data are shown in Table 2; "A" gives the actual percentage absorbed; "B" gives the percentage ratio of each drug to the avreage of the absorption of the six drugs.

Table 3 presents the results in still simpler form, namely, as the means of all the series (calculated from Table 2, B), in ascending order:

TABLE 3.—MEAN ABSORPTION (PER CENT.) OF THE ANESTHETIC HYDROCHLORIDS FROM THE URETHRA

	Per Cent.
Apothesin	89.6
Beta eucain	89.6
Novocain	101.0
Cocain	103.5
Tropacocain	108.6
Alypin	115.2

EFFECT OF CONCENTRATION AND DOSE

In a series of experiments the absorption of the 3 per cent. solutions was compared with that of an equal volume of 10 per cent. solution, using therefore three and one-third times the concentration and three and one-third times the dose.

The results are shown in Table 4. The data on cocain are from experiment 3, the others from experiment 4. The solutions were always left for twenty minutes in the urethra, except when specially noted.

The results show that the concentrated solutions are absorbed much better: the amount absorbed is greater, not only absolutely, but even in percentage. Increasing the dosage and percentage three and one-third times, the amount absorbed was increased, on the average, four and one-fourth times.

TIME OF SOJOURN

Observations on this were made in Experiment 2 and are shown in Table 5. It is seen that the absorption is most rapid in the first ten minutes; but it continues to a fair degree beyond this period.

TABLE 4.—COMPARATIVE ABSORPTION OF 3 PER CENT. AND OF 10 PER CENT. SOLUTIONS OF THE ANESTHETIC HYDROCHLORIDS

	3 per Cent.			10 per Cent.		
	Injected, Mg.	Absorbed, Mg.	Percentage	Injected, Mg.	Absorbed, Mg.	Percentage
Cocain..........	120	26.2	21.8	400	250.4	62.6
Novocain........	90	69.4	77.1	300*	200.2	66.7
Tropacocain.....	90	46.3	55.6	261†	189.7	71.9
Alypin...........	90	41.5	46.0	300	158.8	52.9
Beta eucain......	90	47.8	53.1	300‡	214.4	71.5
Apothesin........	90	39.7	44.1	300	145.4	48.5
Average.................		45.2	49.6		193.2	62.4

* The 10 per cent. novocain was left in the urethra for twenty-six minutes, instead of twenty minutes.

† The tropacocain solution was 8.7 per cent. instead of 10 per cent.

‡ Beta eucain lactate was used in the 10 per cent. solution.

TABLE 5.—INFLUENCE OF TIME ON URETHRAL ABSORPTION OF COCAIN HYDROCHLORID

Time in Urethra, Minutes	Absorbed, per Cent. Injected
10	42.6
20	42.7
30	59.7
40	61.0

INFLUENCE OF ALKALI

Previous papers of this series showed that the addition of sodium bicarbonate increases the efficiency of the local anesthetics when applied to mucous membranes, presumably by favoring penetration. However, it was pointed out that this differs for different mucosae. The direct study of the absorption shows that, in the case of the urethra, sodium bicarbonate does not modify the absorption to a marked degree. The results are shown in Table 6.

TABLE 6.—INFLUENCE OF ALKALI ON THE ABSORPTION

		Per Cent. Absorbed	
Experiment	Drug	Without Alkali	With Alkali
3	Cocain	21.8	32.6
4	Novocain	77.1	75.8

The solutions were freshly made by mixing the 10 per cent. solutions with sufficient of 1 per cent. sodium bicarbonate, so that the final mixture contained 3 per cent. of the anesthetic and 2/3 per cent. of the bicarbonate. This produced a precipitate in the cocain, not in the novocain.

CONCLUSIONS

The absorbability of the hydrochlorids of the local anesthetics from the dog's urethra is in the ratio[2] of: apothesin and beta-eucain = 86.6; novocain = 97.6; cocain = 100; tropacocain = 105.0; alypin = 111.3.

The rate of absorption increases with the dose and concentration; not only absolutely but also per cent. For instance, increasing the dose and concentration three and one-third times increases the absorption four and one-fourth times.

The absorption is most rapid in the first ten minutes, but continues quite markedly even after this time.

The absorption is not materially hastened by rendering the anesthetics alkaline.

These conclusions apply only to the urethra. They should not be transferred to other mucous membranes without direct investigation.

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[2] These numbers are based on Table 3, but are reduced to cocain = 100.

THE PREVENTION OF SIMPLE GOITER IN MAN *

SECOND PAPER

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AND
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(From the Archives of Internal Medicine, July, 1918, p. 41)

In Article I[1] we gave a brief review of the experimental work on which the assertion is based that simple goiter is probably the easiest of all known diseases to prevent. We gave a survey of the incidence and types of thyroid enlargement in the schoolgirls of Akron (Ohio) from the fifth to the twelfth grades, inclusive, and the plan of prevention used.

The plan in operation was arranged from the standpoint of simplicity, practicability, economy and the possible scientific value of the data obtained. First, a census of the condition of the thyroid gland was taken of all girls between the fifth and twelfth grades, inclusive, and the findings recorded on individual cards. This card will be used throughout the whole series of observations, the condition of the thyroid noted each year and a record of all treatment kept on the back of the card.

Number..................................	Date..................................
Name..................................	School..................................
Age................ Weight............	Physical Development..............
Grade..................................	Class Standing....................
Tonsils Adenoids......................	....
Thyroid............	1..
Simple..................................	2..
Adenomas..............................	3..
Thyroid tract..........................	4..
Duration..	
Remarks..	

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* From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University.

[1] The Prevention of Simple Goiter in Man, Jour. Lab. and Clin. Med., **3**: 40, 1917.

It was planned that the thyroid examinations should be made by a single examiner in order to make the standard used constant, and the data obtained uniform. At the time of the first examination, however, it was clearly foreseen that Dr. Marine would be called to military duty. Therefore, in order that the junior author should be trained to examine and classify the cases in the November examination as nearly as possible precisely in the same way as was done in the April examination, the April examination was made by both authors conjointly. It is obvious that much of the value of observations of this kind must depend on the uniformity of the methods and classification.

The thyroid glands were examined from the standpoint of Normals, Slight, Moderate and Marked Enlargements, Adenomas, Persistent Thyroglossal Tracts, and the girls for gross manifestations of myxedema, or exophthalmic goiter. No obvious case of either myxedema or exophthalmic goiter has been found.

Under *Normal* we have included all glands (*a*) which are not visible as a bulging of the skin across the trachea; (*b*) having a barely detectable band of thyroid tissue across the trachea on palpation, and (*c*) absence of a well defined thyroglossal stalk (so-called pyramidal process).

The cases with *Enlarged Thyroids* have been divided into three arbitrary groups: (1) Slight, (2) Moderate and (3) Marked Enlargement. Under *Slight Enlargement* we have grouped those cases with (*a*) visible bulging of the skin over the thyroid isthmus (except in very stout children), and (*b*) a widened and thickened isthmial band or mass on palpation. If the isthmus can not be seen or felt, it can be felt by having the child swallow while the finger or thumb is held against the trachea just below the cricoid cartilage. Under *Moderate Enlargement* we have grouped those with gross deformity, bulging of the neck laterally from the enlarged lobes and marked bulging of the skin anteriorly from the enlarged isthmus. Under *Marked Enlargement* we have grouped those cases with excessive deformity.

Analysis of Thyroid Examinations.—In April, 1917, 3,872 girls of the fifth to twelfth grades, inclusive, were examined and the general results are given in Table 1. In November, 4,415 girls of the fifth to twelfth grades, inclusive, were examined and the new records are given in Table 1. The new records each year will be classed in this table.

TABLE 1.—CONDITION OF THYROID GLAND

	Normal		Slight Enlargements		Moderate Enlargements		Marked Enlargements		Adenomas	
1917	Pupils	%	Pupils	%	Pupils	%	Pupils	%	Pupils	%
April	1,688	43.59	1,931	49.88	246	6.35	7	0.18	29	1.01
Nov.*	831	47.00	820	46.20	121†	6.80				

* Fifth grade girls and girls who entered the Akron schools since April, 1917.

† This number is greater than it would be if only fifth grade pupils were examined; two large schools, one a high school, accidentally destroyed all records of those girls not taking treatment, and therefore had to be recorded as new records.

For the prophylactic treatment we selected sodium iodid on the grounds of economy and ease of administration. Regarding the amounts that should be given, we had no data except from animal experimentation, and as we have pointed out repeatedly, exceedingly small amounts of iodin are effective. In all our dispensary work with children we have used either syrup of hydriodic acid or syrup of ferrous iodid, in 1 c.c. doses daily for two or three weeks, repeated twice yearly. The dosage is much less than the therapeutic dose of iodids in other instances in which they are so extensively used, and there can be no reasonble doubt that the action is very different.

We, therefore, arbitrarily selected to use 2 gm. sodium iodid, given in 0.2 gm. doses each school day, for each pupil in the fifth, sixth, seventh and eight grades; and 4 gm. in 0.4 gm. doses each school day for each pupil in the ninth, tenth, eleventh and twelfth grades. These amounts were given in May, 1917, but in November, 1917, we gave 2 gm. to each pupil from the fifth to twelfth grades, inclusive, since this amount in the year's use gave such definite results. As was pointed out in the previous paper, it was thought likely that the dose would be materially reduced. This amount (*2* gm.) will be given again in April, 1918. The treatment is given at the school by the teacher or principal and the number of doses recorded. A record is kept, both of those who take the treatment and of those who do not, and all pupils are to be examined annually and the thyroid conditions recorded.

A complete reexamination of all girls from the fifth to twelfth grades was made in November, 1917. There were 1,772 new records (Table 1). These include (*a*) this year's *fifth* grade; (*b*) pupils of all grades above the fifth entering Akron schools since April, 1917, and (*c*) all the girls in two schools (*not* taking treatment) whose records had been accidentally destroyed (see footnote, Table 1). All those previously examined were classed either as taking prophylactic treatment or not taking prophylactic treatment. The results are summarized in Table 2.

TABLE 2.—SUMMARY

	Pupils Taking Prophylactic Treatment		Pupils Not Taking Prophylactic Treatment	
	Pupils	%	Pupils	%
Thyroids remained normal...........	283	100.0	637	74.0
Increased from normal to slight goiter	0	0.0	259	26.0
Small goiters (unaltered)............	287	66.0	759	87.0
Small goiters (disappeared).........	141	33.5	10	1.2
Small goiters (increased).............	2	0.5	103	11.8
Large goiters (unaltered)............	34	66.7	106	95.5
Large goiters (decreased)............	17	33.3	5	4.5
Total..............................	764		1,879	

Total number of girls examined 4,415

It will be seen that not a single pupil in whom the thyroid was normal last year and who took iodin, showed any enlargement, while of those not taking iodin, 26 per cent. showed definitely enlarged thyroids —some moderately large goiters. Even more than a prophylactic action is shown in the results—just one third of the goiters marked "small goiters" disappeared; and one third of those marked "moderate goiters" showed a decrease of 2 cm. or more. Accordingly, a distinct therapeutic effect is clearly demonstrated.

It was suggested by some physicians that we would have many cases of iodid rash. We spoke of this possibility in every school and asked the principal and teachers to look for symptoms and call the attention of the school nurse and physician to every possible case. There were more than a thousand girls who took the full treatment, and only five developed any noticeable rash. None of these gave any trouble and the condition lasted only three or four days. Four of the girls continued the treatment and paid no attention to it, while the fifth asked to be excused from further treatment and the rash promptly cleared up.

As to the possibility of producing symptoms of Basedow's disease by giving iodin (in small doses) to a large number of girls indiscriminately, we can say that we have not seen a single instance in which any sign of such an effect was produced.

The earnestness with which the school girls have taken up the prophylaxis of goiter is encouraging for the practical application of this work. It is entirely elective on the part of the girls, and last year 1,080 girls finished the treatment. This year approximately 2,000 girls are taking the treatment.

SUMMARY

1. Simple goiter can be prevented by the administration of small amounts of iodin.

2. One third of the cases of uncomplicated simple goiter disappear or are markedly decreased by the use of a small amount of iodin, given internally.

3. There is no danger of producing a toxic condition ("Basedow's disease").

4. A very small proportion of the cases (at most 0.5 per cent.) may develop an iodid rash, which promptly clears up on stopping the treatment.

We wish to thank Prof. H. V. Hotchkiss (Superintendent of the Public Schools), the Board of Education and the Principals of the several schools; through whose excellent organization and system, as well as the interest in the problem, the work was made possible.

DISTINCTIVE PROPERTIES AND CLASSIFICATION OF ORGANIC AND COLLOIDAL SILVER COMPOUNDS *

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(From the Journal of the American Pharmaceutical Association, August, 1918, p. 677.)

CONTENTS.

1. Objects.
2. Tests and Data.
3. Classification.
4. Distinctive Properties of the Drugs.

1. OBJECTS

A large number of silver protein compounds are now on the market, and it is desirable to classify these in a logical manner. Collargol, Argyrol and Protargol, for instance, evidently represent quite distinct types. May the other compounds be arranged under these three types, or are further groups necessary? What should be the criteria for a classification? The following investigation is intended as a partial and tentative answer to these questions. It includes the application and the discussion of a series of tests and data to the silver protein and colloidal silver compounds accepted in "New and Non-official Remedies"[1] and of a few which have not yet been accepted.[2]

2. TESTS AND DATA

Condition of the Silver.—Silver ions precipitate ordinary proteins and are therefore highly irritant to the tissues. Compounds containing their silver in non-

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* This investigation is partially supported by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

[1] Electrorgol was not included, because of its very low silver content.

[2] Silver proteinate, Heyden; Silver nucleinate and proteinate, Roche; Solargentum, Squibb; and Silvol.

ionized or "masked" or "colloidal" form do not precipitate and therefore largely avoid the irritation. This is the main object in the therapeutic use of these silver compounds. Various precipitant reactions may be used as tests for free silver ions. The most convenient, perhaps, is the precipitation of chlorides. A modification of this will be described later.

It may be stated now that all but two of the preparations contain all of their silver in non-ionized form; and these two (Albargin and Roche Silver Nucleinate) contain only a small, but not negligible, amount of ionized silver, presumably as a contamination.

TABLE I.—TOTAL SILVER CONTENT AND PROTEIN COMPONENT

Drugs (Arranged in Order of Silver Content)	Claimed Percentage of Sliver	Claimed Protein Base
Collargol................	78	
Cargentos................	50	
Argyrol.............. ...	20-25	Vitellin (in reality probably denatured egg albumin)
Squibb Silver Prot.	19-23	Gelatin
Roche Silver Nucl.	20	Probably denatured serum albumin
Solargentum...........	about 20	Gelatin
Silvol................... ..	20	?
Sophol....................	20	Methylen nucleinic acid
Albargan.................	15	Gelatin
Novargan................	10	Albumin (?)
Protargol.................	8.3	Albumin (?)
Heyden Silver Prot.	8.3	Albumin (?)
Roche Silver Prot.	8.0	(?)
Hegonon..................	7	Albumose
Argonin..................	4.28	Casein

Total Silver Content.—The quantities claimed by the manufacturers are shown in Table I. It will be noted that the content of the three typical compounds, collargol, argyrol and protargol, are distinctive; and the most of the other compounds approach one or the other of these types. It remains to be seen whether this coincides with other distinctive properties. Actually this is very generally the case.

Protein Component.—This is not very important, since it is practically a mere vehicle. Some of the manufacturers prefer to keep its nature secret, as a valuable manufacturing asset. Others follow the much more reprehensible course of giving misleading names to their proteins. This abuse is not easily stopped, since there is no known method for identifying the protein in the finished product. The data, as far as known, are shown in Table I.

Antiseptic Efficiency.—The silver-protein compounds are used almost exclusively on mucous membranes, for antiseptic or astringent, or for demulcent effects. A classification according to antiseptic efficiency would be desirable, but does not appear feasible at present. The results of culture experiments cannot be transferred with confidence to clinical conditions; and no satisfactory technic has been developed for life tests.

Irritation.—This is perhaps the most important criterion for a clinical classification. It is easily judged by placing a few drops of the 1 : 10 watery solutions[3] in the nostrils, and in case of doubt also in the conjunctival sac. The method is subjective and incapable of absolute quantitative expression. Nevertheless the differences are sufficiently striking to permit arrangement into three groups, as in Table 2.

The data refer to a 1 : 10 aqueous solution in the nostrils. Those which were also tried in the conjunctiva are marked * *.

TABLE 2.—IRRITATION

No Irritation	Slight But Distinct	Marked Irritation
Argyrol	Protargol	Albargin*
Argonin (5%)	Heyden Silv. Prot.	
Silvol**	Cargentos	
Sophol	Silv. Nucl. Roche	
Squibb Sil. Prot.	Silv. Prot. Roche** (very slight*)	
Novargan	Hegonon** (very slight)	
Collargol		
Collargol Old (5%)		
Solargentum		

* Albargin gives a precipitate with sodium chlorid. The filtrate from this appears less irritant. This suggests that the irritation is due largely, if not solely, to free silver ions.

Albumin Precipitation.—This is in a sense an index of astringency. However, it was observed that some nonirritant preparations may, nevertheless, give a pre-

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3. 1: 10 *Solution of the Silver Salts:* 0.5 gm. of the salt is dissolved in 5 c.c. of distilled water. This solution is also used for the egg test, the absorption test, and the color index.

cipitate with albumin. This is most conspicuous with silver. It may be that, in this case, the precipitation is due to a change of the colloidal state of the silver compound rather than in the albumin.

However, the test as elaborated, at least helps to distinguish certain of the compounds that are otherwise closely related. It is also an objective confirmation of the distinct though slight astringent action of all the members of the protargol type (Protargol, Roche and Heyden Silver Proteinate; and Hegonon); and of the marked astringent action of those containing free silver ions (Albargin; Roche Silver Nucleinate).

Technic of the Egg Test.—About one-half inch (about 2 c.c.) of undiluted egg white is placed in a test tube. To this is added 1 c.c. of 1:10 solution of the silver salt. They are thoroughly mixed by shaking, then allowed to stand for about 15 minutes, and then diluted with 15 c.c. of water. If this is too deeply colored, more water may be added. (The egg-white must be added undiluted, as stated.) The results are shown in Table 3.

TABLE 3.—ALBUMIN PRECIPITATION

- The mixture remains clear:
  - Argyrol
  - Squibb's Silver Prot.
  - Solargentum Squibb
  - Collargol
- The mixture is nearly clear:
  - Argonin
  - Sophol
  - Silv. Prot. Roche
  - Hegonon
- The mixture is distinctly turbid:
  - Protargol
  - Novargan
- There is a distinct precipitation:
  - Heyden Silver Prot. (curdy)
  - Cargentos (very fine)
  - Silvol (very fine)
  - Silv. Nuclein Roche (curdy)
- Abundant curdy precipitate:
  - Albargin (2 samples)
- Unsuited for test:
  - Collargol, old sample

Adsorption.—The difference between the preparations are presumably based largely on differences in their colloidal state. It was attempted to demonstrate them by the behavior toward adsorbents. "Permutit" was found to have no distinct effect on argyrol solutions and very little on protargol. "Loyd's reagent," however, showed characteristic differences in the decolorization for the members of each group, although it had no apparent relation to a broader classification.

Technic.—One c.c. of a 1 : 10 solution of the silver salt is diluted with 10 c.c. of distilled water and shaken with 0.5 gm. of "Lloyd's Reagent" (Hydrated Aluminum Silicate), allowed to stand for a few minutes, and filtered. The color of the filtrate is noted.

The degree of decolorization is shown in Table 4.

TABLE 4.—ADSORPTION OF COLOR BY "LLOYD'S REAGENT"

Practically Completely Destroyed	Considerably Destroyed*	Little, if Any, Decolorized
Argyrol	Protargol	Cargentos
Albargin (2 samples)	Silv. Prot. Heyden	Collargol
Argonin	Novargan	Sophol
Collargol, old sample	Silvol	Solargentum Squibb
Silv. Nuclein Roche	Hegonon	Silv. Prot. Squibb
Silv. Prot. Roche		

* These are arranged in order of decreasing adsorption, the protargol filtrate being the least colored, and hebonon the most.

To a portion of the filtrate (further diluted if too dark) is added a drop of 25 per cent. sodium chlorid, to reveal free silver ions. (Silver nitrate, even in very dilute solution, is not adsorbed by Lloyd's Reagent.)

Free Silver Ions.—The sodium chlorid test is conveniently applied to the filtrate from the Lloyd's Reagent. The test was found negative with all but Albargin (two samples) and with the sample of Roche's silver nucleinate.

The presence of free silver ions renders the solutions irritant, and therefore defeats the special object of the protein compound.

Solubility Behavior.—Table 5 shows that nearly all the protein silver compounds dissolve promptly and completely, at least to 25 per cent., when placed in contact with water. The most conspicuous exception is Argonin, which dissolves slowly and imperfectly, like dried serum.

A strikingly abnormal solution behavior is also shown by an old sample of Collargol, which could not be suspended in water. This had been kept in an amber, glass-stoppered bottle probably for over ten years.

TABLE 5.—SOLUBILITY BEHAVIOR

The following observations were recorded in making the 25% solutions:

- Solution complete and very prompt:
  - Argyrol
- Solution complete and prompt:
  - Protargol
  - Roche's Silv. Nucleinate
  - Squibb's Silv. Prot.
  - Silvol
- Solution complete and fairly prompt:
  - Albargin
  - Hegonon
  - Heyden Silv. Prot.
  - Novargan
  - Sophol
  - Solargentum
- Solution fairly prompt, but not quite complete:
  - Roche Silv. Prot.
- Undissolved, sticky mass, 10% also incomplete, 1% is turbid:
  - Argonin
- Permanent colloidal solution when titrated in water:
  - Cargentos
  - Collargol
- Not miscible with water:
  - Collargol, old sample

Specific Gravity of 25 Per Cent. Solutions.—Some ophthalmologists use the protein-silver compounds, especially the Argyrol type, for their specific gravity rather than for their antiseptic effect, the idea being that the heavier silver solutions would displace the secretions, pus and bacteria by gravity. Some such mechanical action would doubtless occur, if the patient is placed in the proper position; but if this has any value it would be obtained equally well, and much more cheaply, by a 25 per cent. solution of acacia.

TABLE 6.—SPECIFIC GRAVITY OF 25 PER CENT. SOLUTIONS

Collargol	1.230	Albargin	1.112
Cargentos	1.166	Heyden Silv. Prot.	1.108
Solargentum	1.159	Protargol	1.104
Argyrol	1.147	Roche Silv. Prot.	1.100
Acacia	1.137	Hegonon	1.093
Silvol	1.137	Novargan	1.090
Sophol	1.132	Squibb Silv. Prot.	1.088
Roche Sil. Nucl.	1.123		

Argonin is not soluble to 25 per cent. A 2.5 per cent. solution has the specific gravity, 1.010.

The "25 per cent." solutions were made by dissolving 0.5 gm. of the substance in 1.5 c.c. of distilled water.

The specific gravity was determined by weighing 1 c.c. in an Ostwald pipet, at 20 C. The results are shown in Table 6, in descending order.

Viscosity of 25 Per Cent. Solutions.—This has no special relation to the natural groups, and is probably without clinical significance. It was estimated by the time of outflow from a 1 c.c. Ostwald pipet (from the 1 c.c. mark, to the junction of bulb and beak), at 20 C. The results shown in Table 7 are arranged in descending order.

TABLE 7.—VISCOSITY OF 25 PER CENT. SOLUTIONS

Name	Seconds Required for Outflow (Water = 40 Sec.)	Viscosity (Water = 1)
Hegonon	285	7.125
Sophol	180	4.125
Albargin	174	4.350
Squibb Silv. Prot	135	3.375
Cargentos	120	3.0
Roche Silv. Nucl.	102	2.550
Protargol	75	1.875
Novargan	71	1.775
Argyrol	54	1.350
Heyden Silv. Prot.	53	1.325
Silvol	50	1.250

Acacia: The 25 per cent. solution flowed too slow for measurement. A 10 per cent. solution (1 gm. + 9 c.c.) flowed in 165 seconds, corresponding to a viscosity of 4.125.

Argonin, Collargol, Solargentum, and Roche's Silv. Prot. did not dissolve completely, and thus could not be measured.

APPEARANCE OF THE COMMERCIAL PRODUCTS: This is only of incidental interest. The specimens may be arranged as follows:

Coarsely granular
  Argyrol (dull black masses)
  Collargol, old sample (dull gray-black masses)
  Collargol, fresh sample (brilliant steel blue granules)
  Solargentum (bright black granules)
Bright black scales:
  Cargentos
  Silv. Nucl. Roche
  Silv. Proc. Squibb
  Silvol

Coarse Powders:
- Albargin (appearance of dried albumen)
- Argonin (buff)

Fine powders: (arranged in order of decreasing depth of color)
- Heyden Silv. Prot. (dark red to dark chocolate tint)[4]
- Sophol (chocolate)
- Protargol (reddish brown)[5]
- Roche Silv. Prot. (milk chocolate)
- Hegonon (milk chocolate)
- Novargan (buff)

Depth and Color of Transparency of 1:1000 Solutions.—This is a rough but useful aid to identification. The clearness or turbidity to reflected light appears related to the natural groups, the colloidal silver and the protargol type being generally turbid, whereas the argyrol type is generally clear.

TABLE 8.—APPEARANCE OF 1 : 10,000 SOLUTIONS

	Solution Clear both With Transmitted and Reflected Light	Clear With Transmitted Light, but Turbid by Reflected Light	Turbid both With Transmitted and Reflected Light
Color-very deep....	....	Cargentos	Collargol
Color deep.........	Argyrol Silvol Solargentum Silv. Prot. Squibb	Silv. Nucl. Roche	
Color moderate....	Hegonon Protargol* Silv. Prot. Heyden Silv. Prot. Roche	Albargin Sophol	
Partially colored..	Novargan		

Argonin has a different, violet tinge, and is turbid both by transmitted and reflected light.

* Different samples of Heyden's Silver Proteinate and of Protargol vary materially in color.

For making the comparisons, 1 c.c. of 1 : 10 solution is added to 100 c.c. of water in a conical "urine-glass." The results are shown in Table 8.

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[4] Different samples of protargol and Heyden's silver proteinate vary considerably in color.

[5] Different Samples of Heyden's Silver Proteinate and of Protargol vary materially in color.

Color Index.—Colorimetric measurements furnish a more accurate quantitative expression of the color intensity. This is fairly characteristic for the groups, the collargol group standing the highest, the argyrol group intermediate, and the protargol group lowest. There are, however, individual exceptions. The color intensity increases with the silver content, but not in simple proportion.

Technic.—The 1 : 1,000 to 1 : 50,000 solutions are compared with tenth normal potassium dichromate in a Duboscq colorimeter, set at 20. The tints do not match well, but sufficiently so to express the great difference in intensity.

The simple color index expresses the ratio of the color intensity of the 1 : 1,000 solution of the silver compound, to the tenth-normal dichromate solution.

The silver color index is the simple index, divided by the percentage of silver in the compound. The results are shown in Table 9, with the compounds arranged in descending order of silver-color index.

TABLE 9.—COLOR INDEX

Name of Substance	Dilution	Match of Tint	Reading	Simple Index	Silver Index
Collargol	50,000	Fair	16	62.5	0.80
Heyden Silv. Prot. (aver.)	......	.....	..	3.14	0.39
Cargentos	10,000	Good	12	16.7	0.33
Silvol	10,000	Good	31	6.5	0.30
Roche Silv. Nucl.	10,000	Fair	34	6.0	0.30
Argyrol	10,000	Fair	48	4.2	0.20
Squibb Silv. Prot.	10,000	Good	47	4.3	0.20
Protargol (average)	......	......	..	0.80	0.10
Roche Silv. Prot.	1,000	Fair	28	0.72	0.09
Hegonon	1,000	Poor	50	0.4	0.06
Sophol	1,000	Poor	40	0.50	0.025
Albargin	1,000	Very poor	55	0.36	0.024

3. CLASSIFICATION OF THE PROTEIN AND COLLOIDAL SILVER COMPOUNDS

The study of the preceding data has led to the compilation of the properties most useful for grouping. These are shown in Table 10.

TABLE 10.—CLASSIFICATION AND SIGNIFICANT PROPERTIES OF PROTEIN AND COLLOIDAL SILVER COMPOUNDS

Principal types	"Collargol"	"Argyrol"	"Protargol"	"Free Silver"	"Pale"
Members	Collargol Cargentos	Argyrol Squibb Silv. Prot. Sophol Silvol Solargentum (?)	Protargol Roche Silv. Prot. Heyden Silv. Prot. Nucl. Hegonon	Albargin Roche Silv.	Norvagan Argonin
Silver content	50 to 78	20 to 25	7 to 8.5	*	4 to 10
Irritation	*	None	Slight but distinct	Marked	*
Albumin precipitation	*	*	More or less	Positive	*
Adsorption of color	None	*	More or less	*	*
Free silver	None	None	None	Present	None
Specific gravity of 25 per cent. solution	1.160 to 1.230	1.132 to 1.147	1.093 to 1.108	*	*
Transparency of 1:1,000 solution by reflected light	*	Clear (except Sophol)	Clear	Turbid	*
Simple color index	16.7 to 62.50	4.2 to 6.5 (except Sophol, 0.50)	0.37 to 1.75	*	Very low
Silver color index	0.33 to 0.80	0.20 to 0.30 (except Sophol, 0.025)	0.044 to 0.20	*	Very low

* = inconstant or not characteristic.

4. DISTINCTIVE CHARACTERS OF THE INDIVIDUAL DRUGS

These are arranged by groups and shown in Tables 11 to 14.

TABLE 11.—DISTINCTIVE CHARACTERS OF THE ARGYROL TYPE

	Members				
	Argyrol	Squibb's Silv. Prot.	Sophol	Silvol	Solargentum
Silver per cent. (claimed)	20-25	19-23	20	20	About 20
Irritation by 10 per cent. sol.	None	None	None	None	None
Albumin	No precipitate	No precipitate	Nearly clear	Distinct precipitate	No precipitate
Lloyd's reagent, filtrate	Complete decolorization	Not decolorized	Not decolorized	Consid. decolorization	Deeply colored
Free silver (chlorid test)	None	None	None	None	Absent
Solution	Very prompt	Prompt	Fairly prompt	Prompt	Permanent
Sp. Gr. of 25%	1.147	1.088	1.142	1.137	1.159
Viscosity of 25%	1.350	3.375	4.5	1.250	
Appearance	Dull black mass	Bright black scales	Fine chocolate powder	Bright black scales	Brilliant black granules
Color of 1:1,000 solution	Deep	Deep	Moderate	Deep	Deep
Transparency by reflected light	Clear	Clear	Turbid	Clear	Clear by both transmitted and reflected light
Sample color index	4.2	4.3	0.50	6.5	
Silver color index	0.20	0.20	0.025	0.30	5.0
Nature of protein base	Denatured albumin	Gelatin	Methylen nucleinate acid	?	0.25

TABLE 12.—DISTINCTIVE CHARACTERS OF THE COLLOIDAL SILVER TYPE

Members	Cargentos	Collorgol (recent sample)
Per cent. silver (claimed)	50	78
Irritation by 10% sol.	Slight, but distinct	None
Albumin	Fine precipitate	No precipitate
Lloyd's reagent, filtrate	Deeply colored	Deeply colored
Free silver (chlorid test)	Absent	Absent
Solution	Permanent	Permanent
Spec. Grav. of 25%	1.166	1.230
Viscosity of 25%	3.0	
Appearance	Bright black scales	Brilliant blue granules
Color of 1:1.000 sol.	Very deep	Very deep
Transparency	Clear by transmitted light, turbid by reflected	Turbid, both with transmitted and reflected
Simple color index	16.7	62.5
Silver color index	0.33	0.80

TABLE 13.—DISTINCTIVE CHARACTERS OF THE PROTARGOL TYPE

Members	Protargol	Roche Silv. Prot.	Heyden Silv. Prot.	Hegonon
Silver, per cent. (claimed).....	8.3	8.0	8.3	7.0
Irritation by 10% sol.	Distinct	Very slight	Distinct	Very slight
Albumin........................	Turbid	Nearly clear	Almost clear to curdy precipitate	Nearly clear
Lloyd's reagent, filtrate	Considerably decolorized	Completely decolorized	Considerably decolorized	Somewhat decolorized
Free silver (chlorid test).......	None	None	None	None
Solution........................	Prompt	Limited	Fairly prompt	Fairly prompt
Sp. Gr. of 25% sol.	1.104	1.100	1.108	1.093
Viscosity of 25% sol.	1.875	......	1,325	7.125
Appearance......................	Reddish brown, fine powder	Fine, milk chocolate powder	Fine, red to dark chocolate powder	Fine, milk chocolate powder
Color of 1:1,000 sol.	Moderate	Moderate	Moderate to deep	Moderate
Transparency by reflected light	Clear	Clear	Clear to turbid	Clear
Simple color index.............	Av., 0.80 (0.37, 0.72, 0.77, 1.25)		Av., 3.14 0.96, 0.4, 1.75, 6.7)	
Silver color index..............	Av., 0.10 (0.044, 0.09, 0.09, 0.15)		Av., 0.39 (0.12, 0.06, 0.20, 0.80)	
Nature of protein base........	?	?	?	Albumose

TABLE 14.—DISTINCTIVE CHARACTERS OF SUNDRY TYPES

Members	Atypical Novargan	Atypical Argonin	Free Silver Ions, Albargin	Free Silver Ions, Roche Silv. Nucleinate
Silver, per cent. (claimed).....	10.0	4.28	15.0	About 20
Irritation by 10% sol.	None	None (5%)	Marked	Distinct
Albumin........................	Turbid	Nearly clear	Abundant curdy precipitate	Curdy precipitate
Lloyd's reagent, filtrate.......	Considerably decolorized	Completely decolorized	Completely decolorized	Completely decolorized
Free silver (chlorid test).......	None	None	Present	Present
Solution........................	Fairly prompt	Limited and gummy	Fairly prompt	Prompt
Sp. Gr. of 25% sol.	1.090	......	1.112	1.123
Viscosity of 25% sol.	1.775	......	4.35	2.55
Appearance......................	Fine, buff powder	Coarse, buff powder	Coarse powder like dried albumin	Bright, black scales
Color of 1:1,000 sol.	Pale	Pale	Moderate	Deep
Transparency by reflected light	Clear	Turbid	Turbid	Turbid
Simple color index.............	......	......	0.36	6.0
Silver color index..............	......	......	0.024	0.30
Nature of protein base........	?	Casein	Gelatin	?

SUMMARY

The colloidal and silver compounds may be arranged into a few typical groups:

The Collargol Group, comprising Collargol and Cargentos.

The Argyrol Group, Argyrol, Squibb's Silver Proteinate, Sophol, and Silvol and Solargentum.

The Protargol Group: Protargol, Roche's Silver Proteinate, Heyden Silver Proteinate, and Hegonon.

The Free Silver Group: Albargin and Roche's Silver Nucleinate.

The Pale Group: Novargan and Argonin.

The distinctive data of these groups are shown in Table 10; the detailed data are reproduced in Tables 1 to 9, arranged acording to the tests; and in Tables 11 to 14, arranged according to the drugs.

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ANTHELMINTICS: THEIR EFFICIENCY AS TESTED ON EARTHWORMS *

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(*From the Journal of Pharmacology and Experimental Therapeutics. October, 1918, p. 129.*)

INTRODUCTION

The war has created a shortage in the supply of the usual anthelmintics, which makes it advisable to utilize the available drugs to the best advantage. Trustworthy and cinvenient tests of efficiency will therefore be especially useful. Several methods of bio-assay have indeed been employed:

1. The direct therapeutic test on man is not always feasible; it is not altogether reliable, because conditions are not easily controlled in human patients; it therefore requires a large statistical material.

2. The therapeutic test on mammals was employed, for instance, by M. C. Hall and Foster.[1] This also suffers, though to a less degree, from the difficulty of exact control of conditions, and therefore requires a larger number of animals.

3. Experiments on the parasites, removed from human or animal subjects and kept alive outside the body under appropriate conditions. The supply of parasites is uncertain, and the maintenance of "normal" conditions is difficult; so that this method has been used mainly qualitatively, especially in ascaris, as in the work of Schroeder,[2] Straub[3] and Bruening.[4]

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* From the Department of Pharmacology of the Medical School of Western Reserve University.

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[1] M. C. Hall and Foster: J. Agric. Res., **12**: 397, 1918.

[2] H. Schroeder: Arch. exp. Path. Pharm., **19**: 291, 1885.

[3] W. Straub: ibid., **48**: 1, 1902.

[4] Bruening: Ztschr. f. Exper. Path. **3**: 564, 1906.

4. Earthworms resemble intestinal "worms" in their reactions toward anthelmintics, and have been used qualitatively for investigating the nature of the action of anthelmintics, for instance by Straub[3] and by Trendelenburg.[5] Yagi[6] proposed the liquefaction on injection of malefern as a specific quantitative test for this drug.

This general similarity of the reactions of earthworms suggested the hope that simple toxicity experiments would furnish valuable data, with certain limitations. This hope has been realized by the results of the following investigations. They show that all anthelmintics are toxic to earthworms. A substance that is not toxic to these worms is therefore scarcely worth further trial; and a substance that is toxic to earthworms is worthy of consideration as an anthelmintic.

It will of course require very extensive investigation before its value as a clinical-anthelmintic is established, for that involves also other considerations—such as the specific efficiency for human parasites in the intestinal tract; and the absence of excessive toxicity or irritation to the patient.

It is not possible, therefore, to deduce the clinical availability or even the clinical efficiency from the order of toxicity to earthworms, except in the sense that drugs that are not toxic to earthworms in fairly high dilutions are probably not anthelmintics whilst those that are effective on earthworms will likely be useful clinically, provided that the conditions of absorption, irritation and toxicity are favorable. This is illustrated by the confirmation of the efficiency of the time-honored pumpkin-seed treatment.

There is a case, however, in which the toxicity to earthworms, and the clinical efficiency should be practically parallel; viz., when comparing closely related drugs; and still more, when comparing different samples of the same drug. For this purpose, the earthworm test is theoretically just as good as the therapeutic test on man or other mammals, and it is of course vastly preferable from a practical standpoint. The importance of this is illustrated in the results on different aspidium and pelletierin preparations.

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[5] Trendelenburg, P.: Arch. f. Exper. Path. u. Pharmakol., **79**: 190, 1915.

[6] Yagi, S.: Ztschr. f. Exper. Med., **3**: 64, 1914.

Finally, the test is ideal from the standpoint of simplicity of technic, and availability of material. Practically unlimited numbers of earthworms of suitable size (1 to 4 inches) may be obtained from a leaf-compost heap, even in midwinter. The factor of seasonable variations has not been determined, since nearly all of the experiments were made within a few weeks.

Experiments on earthworms are also very suitable for class-room work. They may be used in place of the difficultly obtainable ascaris to illustrate Straub's spiral experiment on the repulsive effect of santonin—which, incidentally, is not at all characteristic of that drug.

METHODS OF TOXICITY DETERMINATIONS ON EARTHWORMS

The worms (species not determined) are kept in the laboratory in small crocks, partly filled with leaf mold. When experiments are to be made, the crock is inverted, and the worms, which are mostly at the bottom of the mold, are picked up in mass and dropped in a conical "urine glass"[7] filled with tap water. The solutions to be tested are also made with tap-water and placed in urine glasses, 100 c.c. in each, and five of the worms are introduced. The size of the worms makes remarkably little differences; but if they differ widely, it is advisable to have several sizes in each glass. When only five worms are put into 100 c.c. of water, they keep alive and normally active for more than a week. Much larger quantities, however, become asphyxiated.

In the experimental glasses, the activity of the worms is examined for a few minutes. Irritants generally cause more or less agitation, increased movement, often "whipping." They are again examined after one or two hours, and again on the next day.

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[7] In the case of volatile constituents, glass stoppered bottles of 250 c.c. capacity were used.

Some drugs show peculiarities; thus tannin causes the protrusion of the mouth, strychnin and physostigmin tend to make the worms curl up more or less tightly. When partly depressed, the animals are often club shaped, more marked depression makes them beaded; when dead or nearly so, they become flaccid and soon smeary and diffluent. Little attention, however, was paid to these minor peculiarities.

Notation.—The following letters are used for convenience in denoting the conditions of the animals:

F (fatal), if all the worms are dead or nearly so.

S (subfatal), if the worms were merely depressed or if only a part were dead and the others would presumably survive.

N (normal), if the worms were not visibly affected.

BB (bile salts-bicarbonate) refers to an artificial intestinal juice, viz.: bilein, 0.04 per cent.; sodium bicarbonate, 1 per cent.

Unless otherwise stated, the description refers to the conditions after one day.

The concentrations always refer to per cent.

General Results.—The general toxicity of the substances tried is shown roughly in Table 1. This arranges the compounds, according to their toxicity in five groups. Special attention is called to the following:

1. The high toxicity of mustard oil, explaining the anthelmintic use of the closely related onion and garlic.
2. The high toxicity of copper sulphate, suggests its use in enemas against oxyuris.
3. The anthelmintics that are most efficient clinically also stand high in their toxicity for earthworms, as shown in the second and third columns.
4. Anthelmintics of doubtful clinical efficiency stand low in their toxicity to earthworms, as shown in the fourth column.
5. Substances that are practically nontoxic to earthworms (column 5) are not used as anthelmintics clinically.

Table 2 shows the data in somewhat more detail, and in alphabetical arrangement,

DETAILED DISCUSSION OF THE ANTHELMINTICS

The comparative toxicity is indicated by the class number, the just fatal concentration being: for Class I, 0.0001 to 0.0009; for Class II, 0.001 to 0.009; for Class III, 0.01 to 0.09; for Class IV, 0.1 to 0.9; for Class V, 1 and over.

The tests were generally made with five worms, as described.

1. *Acid, Acetic (Class III).*—0.1 : 100 = F within one hour; 0.01 : 100 = S within one day.

0.1 : 100 produced immediate violent agitation. This cedes promptly to paralysis, the worms being almost dead in three minutes, responding very feebly to pinching. They are found dead in one hour.

TABLE 1.—FATAL CONCENTRATION (PARTS PER 100)

Class I 0.0001 to 0.0009	Class II 0.001 to 0.009	Class III 0.01 to 0.09
Cupric sulphate	Histamin	Acid, acetic
Mercuric chloride	Naphthalen	Acid, hydrochloric
Mustard oil	Oil chenopodium	Ant. potassium tartrate
	Oleoresin aspidum	Areca
	Paradichlorbenzene	Cantharidin
	Pelletierin tannate	Cantharis
	Santonin BB	Granatum (active)
	Thymol	Kamala
		Picrotoxin
		Piperin
		Potassium cyanid
		Pumpkin seed
		Quinin
		Saponin
		Soap
		Sodium carbonate
		Squash seed
		Tannin
1/100 to 1/20 Saturation	**1/20 to 1/10 Saturation**	**1/10 to 1/5 Saturation**
Chenopodium oil	Aspidium oleoresin	Betanaphthol
	Thymol	Camphor
	Turpentine oil	Chloroform
		Ether

Class IV 0.1 to 0.9	Class V 1 and Over
Aspidium (old)	Alcohol (2 × 4)
Bilein	Chalk
Capsicum	Glycerin
Ferric chlorid	Sodium bicarbonate
Gentian extract	Sodium chlorid
Granatum, old	Sodium saccharin
Kusso	
Onion, fresh	
Pepper, black	**Classes IV and V**
Quassia, extract	Aloes
Santonin sodium	Ant. potassium tartrate
Spigelia	

TABLE 2.—LIMIT CONCENTRATIONS TRIED (PER 100 C.C.)

	Over Fatal	Just Fatal	Under Fatal
Acetic acid	0.1	......	0.01
Acid hydrochloric	0.03	......	0.01
Alcohol	4.0	......	2.0
Aloe	......	......	0.1
Ant. potassium tartrate	......	......	0.1
Areca	0.1	......	0.01
Aspidin rhigome, old	......	1.0	0.33
Aspidium oleoresin solution	0.003	0.002	0.001
Beta naphthol	1/5 sat.	......	1/25 sat.
Bilein	0.2	0.1	0.05
Bilein with bicarbonate	......	......	0.08
Camphor	1/5 sat.	......	1/25 sat.
Cantharidin	......	0.01	......
Cantharis	0.1	0.02	......
Capsicum	0.3	......	0.1
Chalk	......	......	sat.
Chenopodium oil	1/20 sat.	......	1/100 sat.
Chenopodium in solution	0.010	......	0.005
Chloroform	1/5 sat.	1/20 sat.	1/50 sat.
Copper sulphate	0.001	......	0.0005
Epinephrin	......	......	0.0005
Ether	1/5 sat.	......	1/20 sat.
Ferric chlorid tincture	1.0	......	0.1
Ferric chlorid salt	0.08	......	0.008
Gentian extract	1.0	......	0.2
Glycerin	......	......	0.01
Granatum, active	0.1	......	0.01
Granatum, old	1.0	......	0.2
Histamin	......	0.001	......
Kamala	0.1	......	0.01
Kusso	0.25	......	0.05
Mercuric chlorid	0.001	......	0.0001
Mustard oil	0.0025	0.0005	......
Naphthalen	0.01	0.002	......
Onion, fresh	1.0	0.2	0.1
Paradichlorbenzene	0.01	0.001	......
Pelletierin tannate	0.005	......	0.0025
Pelletierin tanret	10.0	5.0	1.0
Pepper, black	0.125	......	0.1
Physostigmin	......	......	0.0006
Picrotoxin	......	......	0.01
Piperin	......	0.01	......
Potassium cyanid	0.1	0.03	0.01
Pumpkin seed, peeled	1.0	0.1	0.01
Quassia extract	......	0.5	0.05
Quinin hydrobrom	0.1	......	0.02
Saccharin sodium	......	......	1.0
Saccharose	......	......	1.0
Santonin (solution)	0.01	......	0.001
Santonin sodium	......	0.5	0.2
Saponin	0.1	......	0.01
Soap	0.1	......	0.03
Sodium bicarbonate	......	......	1.0
Sodium carbonate	0.01	0.03	0.01
Sodium chlorid	......	......	1.0
Spigelia	0.5	0.1	0.02
Squash, cold filtrate	0.1	......	0.01
Squash, boiled filtrate	......	2.0	1.0
Strychnin	......	......	0.01
Tannin	0.05	......	0.01
Thymol	1/10 sat.	......	1/30 sat.
Thymol	0.01	0.002	0.0005
Turpentine	1/10 sat.	......	1/30 sat.
Tyramin	......	......	0.02

0.01 : 100 produced immediate agitation. In an hour. the worms were sluggish. Next day they were normal.

Ch. Darwin[8] cites Perrier that acetic acid is highly toxic to earthworms. Crozier[9] studied the retraction time. He found that the efficiency of a given H-ion concentration increases with the power to penetrate cells.

2. *Acid Hydrochloric (Class III)*.—0.03 : 100 = F in 1¼ hours; 0.01 : 100 = N for one day.

0.03 : 100; this experiment was started by placing 1 c.c. of 1 per cent. HCl in the bottom of a urine glass containing 100 c.c. of water. This produced violent repulsive movements. Enough acid was then mixed with the water to make 0.03 per cent. The worms were found dead in one hour.

The toxicity of hydrochloric acid is apparently similar to acetic acid. This toxicity of acid must be an important factor in protecting the stomach against the introduction and development of parasites. Ascaris probably reacts more slowly to poisons, because of its resistant skin; but Schroeder[2] found that 0.8 per cent of H_2SO_4 killed ascaris in about two hours.

3. *Alcohol* (*Class V*).—5 : 100 = F between 1½ hours and 1 day; 4 : 100 = F between 5 hours and 1 day; 2 : 100 = N for 1 day.

5 : 100; no immediate reaction. Progressive depression after 4 minutes. Almost nonreactive and nearly dead in 1½ hours. Dead on next day.

4 : 100; in 2½ hours, feeble and flaccid, one alive. Same in 5 hours. Dead on next day.

2 : 100; practically no change at any time. Slightly depressed after 1 day.

Schroeder[2] found alcohol promptly narcotic to ascaris.

4. *Aloes* (*Class IV or V*).—0.01 : 100 = N for 1 day.

A filtered watery solution was used. There was practically no effect at any time.

Aloes may therefore be considered useless as an anthelmintic. It is doubtful whether it would have a flushing effect even on oxyuris.

5. *Antimony-Potassium Tartrate* (*Tartar Emetic*) (*Class IV or V*).—0.1 and 0.01 : 100 = N for 1 day.

0.1 : 100 had no perceptible effect.

0.1 : 100 caused slight agitation at first. After a day, the animals were perhaps a trifle sluggish, but firm.

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[8] Darwin, Chas.: "The Formation of Vegetable Mould Through the Action of Worms."

[9] Crozier, W. J.: Am. J. Physiol., **45**: 323, 1918.

These results do not indicate a promising degree of anthelmintic effect considering that the drug can only be given in small doses. Hall and Foster[1] state that tartar emetic is commonly used against roundworms in horses. Their own tests on pigs did not give conclusive results.

6. *Areca* (*Class III*).—0.1 and 0.5 : 100 = F within 1 day; 0.01 : 100 = N for 2 days.

This was used as a fresh filtered infusion made from a sample identified by Dr. Henry Kraemer of the University of Michigan, to whom I am indebted for this and for some of the other drugs.

The tests were made in May.

0.5 : 100; alive, but stiff in 2 hours; dead in 25 hours.

0.1 : 100; dead and indifferent in 21 hours.

0.01 : 100; no effect for 2 days.

These results indicate that areca would be a fairly efficient anthelmintic. Hall and Foster[1] state that it is frequently prescribed against ascarids in dogs and tapeworms in poultry. Their own experiments on both kinds of animals were not very encouraging.

7. *Aspidium Oleoresin* (*Class II*).—In general 0.002 to 0.003 : 100 = F in 1 day; 0.001 to 0.002 = S in 1 day.

Technic.—After several preliminary experiments the following procedure was accepted for making the standard tests:

The oleoresin is shaken, and a very small drop (approximately 0.1 c.c. is placed on a small tared filter paper, weighed and rubbed thoroughly with sand in a mortar, adding the BB solution (bilein, 0.04 per cent.; sodium bicarbonate, 1 per cent.),[10] using 50 c.c. of the solution for 0.01 gm. of the oleoresin. This is filtered and represents 2 mg. of the oleoresin per 10 c.c. of the solution.

The dilutions are made up in urine glasses as follows:

0.01 gm. per 100 c.c. = 50 c.c. of the stock solution and water q. s. ad 100 c.c.;

0.005 gm. per 100 c.c. = 25 c.c. of the stock solution and water q. s. ad 100 c.c.;

0.003 gm. per 100 c.c. = 15 c.c. of the stock solution and water q. s. ad 100 c.c.;

0.002 gm. per 100 c.c. = 10 c.c. of the stock solution and water q. s. ad 100 c.c.;

0.001 gm. per 100 c.c. = 5 c.c. of the stock solution and water q. s. ad 100 c.c.

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[10] Probably simple 1 per cent. sodium bicarbonate could be substituted; some comparative experiments showed no essential difference.

Five worms are placed in each glass and observed after twenty-four hours.

A standard oleoresin should kill the worms in 0.002 to 0.003 per cent.—differing probably a little with the season. Until this point is determined, it may be advisable to check the preparation to be tested against a preparation of known strength (that is, one that is fatal with 0.003 per cent. and only depressant with 0.002 per cent. in cold weather).

Influence of Season.—This was tested on one specimen (III), one determination being made in March, another in May. The results indicate a greater susceptibility in May.

In May as follows:

Concentration	March	May
0.003:100	F: practically dead in 2 hours	F within 18 hours
0.002:100	S: in 1 day, one is dead; 4 are curled and depressed, but fairly alive	
0.001:100 0.0005:100		1 lot F within 18 hours 1 lot N within 21 hours N in 21 hours

Comparison of Specimens of Aspidium Oleoresin.—I was fortunate in securing supplies of the fresh and old oleoresin and other preparations through the kindness of Prof. Ed. Kremers of the University of Wisconsin, and from Parke, Davis & Co. Inasmuch as the Wisconsin material was tested in May, and all the other materials in March, the two groups are tabulated separately.

Materials Tested in March.—The following all killed within a day with 0.003 : 100; whilst 0.002 was survived.

Specimen I, an old museum specimen labeled "Benton, Hall & Co., Cleveland." manufactured since 1906.

Specimen II, a recent market specimen, Eli Lilly & Co, "3696 × 48279."

Specimen III, a recent market specimen, furnished by Parke, Davis & Co., and labeled "2229092."

Specimen IV, an old, returned sample, full of sediment and supposedly inactive, furnished by Parke, Davis & Co.[11]

With all of the specimens, 0.003 : 100 greatly depressed the worms in 2½ hours. They were found dead on the following day.

With 0.002 : 100, no effect was noted in an hour.

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[11] I take pleasure in thanking Parke, Davis & Co. for these specimens.

After a day, the animals were very depressed and clumsy, and just alive. Two worms out of the twenty had actually died.

It is remarkable that the old and presumably spoiled specimens were just as active as the fresh oleoresin. This is also borne out by the Wisconsin series, which, although over 8 years old, measured up fully to the present market samples. Evidently, there need be no fear of the deterioration of the oleoresin. The rhizome itself, however, becomes entirely inactive in time, as will be shown later.

Wisconsin Specimens.—These comprise some commercial samples, and a series of preparations made by Mr. Netzel and Dr. Du Mez in the year 1909-1910. They may be divided into several groups.

F = 0.002; N = 0.001. This group includes the following specimens. An ethereal extract prepared by Netzel from green rhizome; and commercial specimens by Eli Lilly & Co. (8609 x 5790"); Parke, Davis & Co.; and Squibb ("312760"). The Netzel and Squibb samples had abundant sediment; those of Parke, Davis & Co., and Eli Lilly & Co. had practically none.

A sample from Stearns & Co. ("63613H") was weak, surviving with 0.005 per cent., but dying with 0.01 per cent. This had no sediment.

Another specimen prepared by Netzel with ether from "good rhizome" containing a sediment, was intermediate. The worms survived 0.003 per cent. and died with 0.005 per cent.

An acetone-oleoresin by Netzel approached the Stearns preparation (survived 0.005 per cent.; died with 0.0 per cent.). The same toxicity was possessed by an alcoholic extract.

A benzine extract (Netzel) was fairly active (survived 0.003 per cent.; died with 0.005 per cent.).

It is again apparent that the oleoresin retains its full activity for at least eight years. As to solvents, the material was inadequate to express an opinion.

The Sediments in Aspidium Oleoresin.—The activity of these was investigated on the old Parke, Davis & Co. specimens, and on one of the Netzel preparations.

Parke, Davis & Co. Specimen IV contained considerable sediment. Microscopically, this consisted of rather large spheroids, of a radiated structure, reminding of leucin.

A toxicity comparison was made between the clear supernatant fluid, and the sediment-rich bottom layer, using in each case 0.003 : 100. The result was the same in both cases: considering depression, but not actual death, in one day.

This shows that the sediment is not greatly more active than the solution; it does not show whether the sediment has any action, since it was contaminated with more or less fluid.

Purified Sediment.—The sediment was then purified by washing it several times with ether. The liquid and sediment are both soluble in ether, but the sediment more slowly. By quick manipulation it is therefore possible to obtain a white or slightly brownish powder, easily soluble in sodium bicarbonate. This could be separated into a rather coarse sandy portion, of which about 0.015 gram were collected, and a fine powder, formed at least partly by evaporation of the ethereal solution of the coarse powder. About 0.05 gram of this fine powder was gathered. Both sediments were tried in increasing concentrations (bilein-bicarbonate vehicle) until 0.01 : 100 was reached, without killing the worms. The fine powder produced no visible results. The coarse powder caused depression, but this might be attributable to incomplete removal of the fluid.

Netzel Deposit.—This also dissolved readily in the bilein-bicarbonate mixture. Its toxicity was just the same as that of the extract from which it separated.

It is safe to conclude that the sediments occurring in some of the oleoresins on standing, are not more actively anthelmintic than the overlying fluid. More probably they have less or no activity, but they were not separated in sufficiently pure form to demonstrate their inactivity.

Effect of Boiling Aspidium Solutions.—This promptly destroys or at least lowers the activity. It was tried on two occasions, boiling one-fourth hour each time; once 0.003 in one-tenth BB; the other time with 0.005 in one-fourth BB. In neither case was there any visible effect in one day. In the unboiled solutions, the worms were practically dead in two and one-half hours, and quite dead in one day (Sample III).

Literature of Biologic Tests for Aspidium.—W. Straub[3] observed that Taenias survive for a considerable time when kept under suitable conditions in Bunge's solution, but that they die within two hours if 0.01 per cent. filicic acid or aspidin had been added. He also found that invertebrates generally are very susceptible to aspidium. This he attributes to direct paralysis of the muscles.

He used an earthworm test similar to the present for determining the relative efficiency of the aspidium constituents. In this connection he found filicic acid to be much more toxic than albaspidin or flavaspidic acid.

Finally he observed that the injection of 0.1 mg. of filicic acid into a large earthworm killed in six or eight hours, with local liquefaction. (I do not know whether this liquefaction is at all characteristic of aspidium or whether it would not follow any locally toxic agent.) This liquefaction test was later proposed by Yagi[6] as a quantitative method (Fuehner[12]). I do not know whether it has proven successful.

Hall and Foster[1] tried the oleoresin on dogs and cats. They found it highly effective against tenias, doubtful against hookworms, poor against ascarids.

8. *Old Dried Aspidium Rhizome* (*Class V*)—.1 : 100 = just F in 1 day; 0.33 : 100 = N for 1 day. The department museum contains a whole specimen, which was there when I took charge, twenty years ago. It is "well preserved," but has no smell or taste of aspidium. A representative sample was powdered. A 1 per cent. cold macerate in bilein bicarbonate, with or without filtration, caused marked depression in an hour. In a day, the worms were at the point of death. A ⅓ per cent. filtrate left the worms quite alive, although somewhat depressed.

The weak anthelmintic action may have been due to tannin. The characteristic efficiency of the fresh drug, as represented in the oleoresin, has evidently been lost with the characteristic flavor, by the drying and age.

9. *Betanaphthol* (*Class III?*).—⅕ saturated = F; 1/25 saturated = N.

This was used only as dilutions of a stock saturated solution. made by triturating the drug with chalk, macerating with water, and filling and diluting as needed. The same method was also used for aspidium oleoresin, camphor, chenopodium oil, chloroform, ether and thymol. A simple chalk solution had no effect on the worms.

One-fifth saturated betanaphthol produced considerable depression in fifteen minutes and one hour. Next day, dead and flaccid.

One-twenty-fifth saturated, no definite effect in a day.

Betanaphthol would therefore appear to be an efficient anthelmintic, although inferior to naphthalen or paradichlorbenzene.

10. Bile Salts[13] (Class IV).—0.2 : 100 = F; 0.1 : 100 = S; 0.05 : 100 = N.

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[12] Fuehner: "Nachweiss der Gifte," pp. 44 and 45.

[13] "Bilein-Abbott" was used.

0.2 : 100 caused agitation on immersion; sluggishness in one hour; dead next day.

0.1 : 100, after one day, two were dead; four alive, but clubshaped.

0.05 : 100 caused slight agitation on immersion. Normal in one day.

Bile salts are therefore distinctly weak anthelmintics. This is, of course, in consonance with the life of the parasites in the intestinal tract.

11. *Bile Salts and Bicarbonate Mixture* (*Class IV*).—0.08 bilein and 1 per cent. sodium bicarbonate : 100 = S; 0.05 bilein and 1 per cent. sodium bicarbonate : 100 = N. 0.08 : 100 of 1 per cent. sodium bicarbonate for one day caused only a doubtful depression. Evidently the bicarbonate does not increase the toxicity of the bile salts. This point was important in connection with the "BB mixture," which was used as an artificial intestinal fluid in many of the experiments. This "BB" contained 0.04 bilein in 100 of 1 per cent. bicarbonate, or about one-third of the fatal concentration. It was generally further diluted after use.

12. *Camphor* (*Class III*).—⅕ saturated = F between 18 and 25 hours; $1/25$ saturated = N for one day.

Camphor was used as dilutions of a saturated solution, made with the aid of chalk.

The one-fifth saturated caused considerable agitation on immersion, followed by great depression in twenty minutes. In one hour, the worms appeared almost dead; after eighteen hours, they were in the same condition. In twenty-five hours, they were dead and beaded.

Camphor thus appears a quite efficient anthelmintic; but efficient doses would probably be dangerous to the patient.

13. *Cantharidin* (*Class III*).—0.01 : 100, practically fatal in one day.

The cantharidin was dissolved in "bilein-bicarbonate," in which it was apparently soluble.

14. *Cantharis* (*Class III*).—0.1 : 100 = F in 1 day; 0.02 : 100 = practically F in 1 day.

0.5 per cent. powdered cantharis was infused with bilein-bicarbonate, macerated over night, diluted with water, and filtered.

0.1 : 100 caused no apparent change in one hour, but death after one day.

0.02 : 100, after one day, three were dead, three others were almost dead, being of fair tone, but clubshaped.

It is interesting that so specialized an irritant should be so highly toxic to the worms. As a practical anthelmintic, it is of course out of the question.

15. *Capsicum* (*Class IV.*)—0.3 : 100 = F in one day; 0.1 : 100 = N in one day.

Capsicum was used as a 0.5 per cent. decoction with bilein-bicarbonate, macerated for a day, filtered and diluted.

0.5 per cent. (not filtered) was fatal in 2½ hours.

0.3 per cent.; normal in one hour; dead and flaccid in one day.

0.1 per cent.; somewhat depressed in one hour; normal for one day.

Pungent condiments like capsicum, pepper, mustard, onion, etc., appear fairly toxic to worms; explaining their use as adjuvants in "worm cures." This will be discussed again under "Synergistic Combination."

16. *Chalk.*—Saturated solution = N in one day.

This was tried because of the use of chalk in making saturated solutions of different soluble drugs.

17. *Chenopodium Oil* (*Class II*).—0.010 : 100 = F in one day; 1/20 saturated = F in one day; 0.005 : 100 = N in one day; 1/100 saturated = N for one day.

Chenopodium oil was tried in several ways, namely, as saturated solutions after trituration with chalk, and as solutions in bilein, and in bilein-bicarbonate mixture.

Chenopodium Oil-Chalk Trituration.—All the dilutions produced considerable agitation on immersion.

Saturated solution: practically dead in twelve minutes; dead in fifteen minutes.

⅕ saturated solution: dead and beaded in one day.

1/20 saturated solution: pale, incoördinated, but lively after five hours; dead in one day.

1/100 saturated solution: normal in one day.

Chenopodium Oil and Bilein.—A stock solution was made by triturating 0.11 gm. of the oil and 0.04 gm. of bilein with filter paper, sand and water, to make 100 c.c. and filtering.

Chenopodium Oil, per Cent.	Bilein, per Cent.	
0.11	0.04	Depressed in 10 minutes, dead, flaccid and diffluent next day
0.011	0.004	Sluggish but fairly normal in 2½ hours. Next day, very weak, clubshaped, but alive
0.0022	0.0008	Normal for 1 day

Chenopodium Oil in Bilein Bicarbonate, Unfiltered.—In this first series the worms were placed in the unfiltered mixture.

Chenopodium Oil, per Cent.	Bilein, per Cent.	Sodium Bicarbonate, per Cent.	
0.13	0.04	1.0	Depressed in 10 minutes; dead and diffluent on next day
0.013	0.004	0.1	After 2½ hours, very sluggish, moderate tone. Next day, dead and flaccid
0.0026	0.0008	0.02	Sluggish and clumsy after 1 day
0.005	0.04	1.0	Alive in 2½ hours; dead in 1 day

Filtered Chenopodium Oil Bilein Bicarbonate Mixture.—0.025 in "BB" immediate marked agitation; after one hour, very depressed; two hours, dead; one day, dead and flaccid.

0.010 in "BB." Immediate marked agitation; after 3½ hours, very depressed, nearly dead; one day, dead and flaccid.

0.005 in "BB." Normal for one day.

Bruening[4] found the toxic concentration for ascarids in Ringer's fluid to be 0.01 to 0.02 per cent. of the emulsified oil. This therefore requires about twice as much as earthworms. In both cases, the effects consist in temporary stimulation, followed by paralysis in a few hours.

Trendelenburg[5] also described the effects of chenopodium on earthworms as initial stimulation followed by paralysis. He considers this similar to santonin.

Hall and Foster[1] found it a reliable anthelmintic in dogs, causing complete disappearance of ascarids.

The clinical results in man have been highly successful.

18. *Chloroform* (*Class III?*).—⅕ saturated = F within 8 minutes; 1/20 saturated = paralyzed within six minutes, but recover in the solution; 1/50 saturated = N for one day.

Chloroform was tried as a saturated solution, made by triturating with chalk, standing, filtering and diluting as needed. All the dilutions depressed at once.

The results indicate that chloroform is an active and prompt anthelmintic. It has been used somewhat in human practice, against all kinds of worms. No doubt exists as to its efficiency, although its absolute safety is questionable.

Hall and Foster[1] found that it did not show up very well in animal experiments.

19. *Copper Sulphate* (*Class I*).—0.001 : 100 = F within one day; 0.0001 : 100 = N for one day.

All the dilutions produced considerable agitation on immersion. 0.01 per cent. was fatal within thirteen minutes.

0.001 per cent.; the worms were alive after 2½ hours, but were found dead and flaccid after a day.

0.0001 per cent.; after a day, the worms showed good tone, although they were very inactive, even on stimulation.

The toxicity of the copper salt is very high, being equaled only by mercuric chlorid, and surpassed by no other drug, as far as tried. Hall and Foster[1] note its use against stomach worms in lambs and found it highly efficient. They found it unsuitable for dogs, because of the emesis; and this would apply with still more force in man. Investigations with nonemetic compounds might be worth while.

The high efficiency of the copper sulphate may make it available as a "worm killer" for walks, lawns, etc.

20. *Epinephrin.*—This was tried for the possible physiologic effects, rather than as an anthelmintic. 0.0005 : 100 produced no visible effect in a day. No further experiments were made.

21. *Ether (Class III?)*—⅕ saturated = F within one day; 1/20 saturated = N for one day.

Ether was used as dilutions of a filtered saturated solution made with the aid of chalk.

The saturated solution depressed at once, and was fatal in less than twenty-one minutes.

The ⅕ saturated solution also depressed at once, the animals appearing almost dead in seven minutes and in one-half hour. However, they recovered in air. Those left in the solution (in a tightly stoppered bottle) were found dead and beaded on the next day.

The 1/20 saturated solution produced no visible effect of any kind for a day.

Ether thus appears a fairly efficient anthelmintic, although it would need to be determined whether an efficient dose is safe.

Hall and Foster[1] observed in dogs a rather slight efficiency against ascaris, none against hookworm, and probably some against tapeworms.

22. *Ferric Chlorid, Tincture (Class IV).*—1 : 100 = F in two hours; 0.1 : 100 = N for one day.

Inasmuch as the alcohol and acidity as well as the ferric chlorid are concerned in the effect, the concentrations are given simply in terms of the U. S. P. tincture.

1 : 100 produced immediately marked agitation; within one hour, the worms were practically dead; in two hours they were quite dead and stiff.

0.1 : 100 showed no effects.

"Tincture of iron" is used locally in enemas against oxyuris. It would evidently be effective if 1 per cent. of the tincture is employed.

However, ferric chlorid is not a very powerful anthelmintic. If the entire activity of the tincture is referred to its ferric chlorid, it would bring this into Class III.

23. *Gentian Extract* (*Class IV*).—1 : 100 = F in one day; 0.2 : 100 = N for one day.

Undiluted Solid Extract.—Contact with a rod smeared with the extract caused prompt repulsion.

1 per cent. solution in water: slight agitation on immersion; appears normal in an hour; dead and flaccid next day.

0.2 per cent.: no definite effect in a day.

Gentian was tried as representing general bitters. It is a weak anthelmintic and would evidently be ineffective by mouth, although it might be efficient locally by enema.

24. *Glycerin* (*Class V*).—A 1 per cent. solution does not produce any perceptible change in a day. Concentrated glycerin causes repulsion, presumably by osmotic irritation.

25. *Granatum, Active Sample* (*Class III*).—0.1 per cent. = F; 0.01 per cent. = N. Old museum sample (Calss IV). 1 per cent. = F; 0.2 per cent. = N.

The active sample was furnished by Henry Kraemer, and was used in May, 1918, as filtered infusion.

0.5 : 100 increased the tone and excitability in two hours; dead on next day.

0.1 : 100; found dead and flaccid in twenty-one hours.

0.01 : 100 (freshly made); were normal for two days.

The museum sample was of uncertain age. It was also used as infusion.

1 : 100 produced violent agitation on immersion and over an hour. In two hours, the worms had calmed down. Next day they were dead and of a "cooked" appearance (doubtless coagulated by the tannin).

0.1 : 100 also produced considerable agitation on immersion and over 1½ hours. Next day the worms appeared normal.

26. *Histamin* (*Class II*).—0.001 : 100 produced some agitation on immersion. After one day, one worm was dead and beaded, the other four were alive. After two days, a second worm had died, leaving three alive.

The rather high toxicity of histamin is interesting in view of its occasional presence in the alimentary canal, for instance, in connection with ptomaine poisoning.

27. *Kamala* (*Class III*).—0.1 : 100 = F in one day; 0.01 : 100 = N in one day.

This was a museum sample of unknown age, used as filtered infusion with a little bilein-bicarbonate.

The tests were made in May.

0.5 per cent. was fatal in two hours.

0.1 per cent.; the worms were lively in one hour, dead in twenty-one hours.

0.01 per cent.; the worms were normal in twenty-one hours.

The results sustain the reputation of kamala as an active vermicide.

28. *Kusso* (*Class IV*).—0.25 : 100 = F in one day; 0.05 : 100 = N in one day.

A museum specimen of unknown age was used as decoction. Agitation occurred on immersion in all the dilutions.

2.5 per cent. killed the worms within forty minutes.

0.25 per cent. killed between 1½ hours and one day.

0.05 per cent.; nearly normal on next day.

Presumably this specimen has deteriorated from age. Fresh samples would doubtless range with the active anthelmintics of Class III.

29. *Mercuric Chlorid* (*Class I*).—0.001 : 100 = F in one day; 0.0001 : 100 = N in one day.

0.001 per cent. produced violent agitation, which, however, subsided promptly. In two hours, the worms appear almost normal. They are dead on the next day.

0.0001 per cent. produced no effect, beyond slight agitation on immersion.

Mercuric chlorid is thus one of the three most active anthelmintics. Schroeder[2] found 0.1 per cent. fatal to ascarids within an hour. It cannot be safely used clinically, because of its high toxicity to mammals.

30. *Mustard Oil* (*Class I*).—0.0025 = F within 1½ hours; 0.0005 = S in one day.

The volatile mustard oil was added as a 0.5 per cent. solution in 50 per cent. alcohol.

0.025 per cent.; momentary agitation; then coil up, become depressed, and die within five minutes.

0.0025 per cent.; immediate strong agitation; slightly depressed in one-half hour; dead in 1½ hours.

0.0005 per cent.; immediate considerable agitation; next day, five very sluggish; two barely alive; three dead.

The mustard oil in 0.0005 per cent. can be smelled and tasted, but is not irritating to the mouth. The very high toxicity of mustard oil is surprising, although it confirms the anthelmintic efficiency of "spiced" foods. It is interesting that the ham sandwich is so often taken with its antidote.

31. *Naphthalen (Class II).*—0.01 : 100 = F in one day; 0.002 : 100 = S for one day.

The solutions were made with the acid of the bilein-bicarbonate mixture.

In 0.01 : 100, the worms were practically dead in three hours; and quite dead on the next day.

In 0.002 : 100, they were practically dead in one day.

In 0.001 : 100, they were much depressed in one day, but alive.

Schroeder[2] found a saturated solution of naphthalen fatal to ascarids within fifteen hours.

It is evidently a very highly effective anthelmintic.

32. *Onion, Fresh (Class IV).*—1 per cent. = F; 0.2 per cent. = practically F; 0.1 per cent. — N.

Fresh onion was macerated in water and filtered.

In 1 per cent., the worms appeared normal in 3½ hours, but were found dead on the next day.

In 0.2 per cent., they were also normal in three hours, and practically dead on the next day.

In 0.01 per cent., they appeared quite normal for a day.

Fresh onion is therefore a fairly efficient anthelmintic, supporting the usefulness of the raw onion and lettuce supper as a preparative for the more toxic anthelmintics.

Darwin[8] found that earthworms are fond of onions as food. Perhaps, however, their fondness is satisfied by small quantities; or it is also possible that the oil had been practically leached out of the onion leaves employed by him.

33. *Paradichlorbenzene (Class II).*—0.01 : 100 = F; 0.001 : 100 = N.

The substance is nearly insoluble in water, so that a watery solution made with 0.01 per cent. produced no change.

When the solubility was increased by the bilein-bicarbonate combination, 0.01 per cent. proved fatal in less than twenty hours.

0.001 per cent. causes no change in one day; the worms were dead in two days.

My attention was called to this substance by an article by Konantz.[14] I am also indebted to him for the specimen. The substance is evidently a powerful anthelmintic, and should be very useful, if it is found safe. This very important point remains to be determined.

34. *Pelletieren Tannate (Class II).*—0.005 : 100 = F; 0.0025 : 100 = N.

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[14] Konantz, W. A.: J. Am. Pharm. Assn., **7**: 341, 1918.

Specimens.—Four samples were used, three of these having been examined chemically by L. E. Warren.

Method.—It was found that these specimens did not dissolve perfectly in water or in bilein-bicarbonate solution. After some preliminary tests, these were abandoned and the following standard method evolved: The pelletierin tannate, 0.02 gm., is dissolved in 100 c.c. of distilled water by the aid of 0.2 c.c. of 1 per cent. HCl, with thorough trituration in a mortar. This stock solution, which must be freshly made, is then diluted as follows:

40 c.c. of stock solution plus 40 c.c. of water equals 0.01 per cent. of pelletierin tannate;

20 c.c. of stock solution plus 60 c.c. of water equals 0.005 per cent. of pelletierin tannate;

10 c.c. of stock solution plus 70 c.c. of water equals 0.0025 per cent. of pelletierin tannate;

5 c.c. of stock solution plus 75 c.c. of water equals 0.00125 per cent. of pelletierin tannate.

There is considerable agitation on immersion; but the final results are counted after one day. Only the limit concentrations need be given in this paper.

Origin of Sample	Fatal	Practically Fatal	Normal
Sample I, an old Lehn and Fink specimen...	0.01	.....	0.005
Sample II, M. C. W.	0.01	0.005	0.0025
Sample III, P. W. R.	0.01	0.005	0.0025
Sample IV, Merck	0.005	.....	0.0025

It will be noted that pelletierin tannate is, in fact, a very powerful anthelmintic, about half as strong as oleoresin of aspidium; that the commercial specimens are fairly uniform; and that they do not deteriorate easily.

Schroeder[2] found the hydrochlorid harmless to ascarids even in 0.4 per cent. concentration.

Hall and Foster[1] found it unsuccessful against tapeworms in cats, but successful in dogs.

35. *Pelletierin Tanret.*—10 per cent. = F; 5 per cent. = S; 1 per cent. = N.

This proprietary worm-killer is marketed as a syrup, which forms clear solutions with water. These following dilutions were employed:

10: 100: in an hour, the worms are curled and depressed; in a day, they are dead and nodular.

5: 100; in an hour, they have massed themselves as a ball at the bottom of the glass. After a day, one is dead, the other four are curled and much depressed.

1 : 100; they are somewhat depressed in 2½ hours; in a day they are alive and curled up.

Pelletierin tanret is essentially a secret preparation. The advertising pamphlet, while skillfully evading a direct statement, creates the suggestion that it is a solution of a salt of pure pelletierin, freed from the other alkaloids of pomegranate.

According to the foregoing results, the anthelmintic efficiency of the tanret syrup is only $^{1}/_{1350}$ (= $^{0.0037}/_{5}$) of that of the commercial dry pelletierin tannate. According to L. E. Warren[15] the ratio of alkaloidal content is about 1 : 25 (0.818 per cent. in the tanret syrup; 17.4 to 22.8 per cent. in the dry tannates). This would indicate that the efficiency of the tanret alkaloids is considerably lower than would correspond to their percentage. This contradicts the contention of the advertisers that their preparation is superior, in that they claim to have eliminated inactive alkaloids from the commercial pelletierin.

The usual dose of the tanret syrup is given as the contents of a bottle, about 30 c.c. containing about 0.25 gm. of free alkaloids. The average dose of the official pellietierin tannate is also stated as 0.25 gm., but would correspond to 0.05 gm. of free alkaloids, that is, one-fifth of the amount in the tanret dose. This represents just about the difference in anthelmintic efficiency.

In brief, then, the official dose (0.25 gm.) of any of the commercial brands of the pharmacopeial pelletierin tannate, dissolved in 30 c.c. (an ounce) of syrup of citric acid, would be just as pleasant, much cheaper, and probably about four times as effective as the average dose of the secret pelletierine tanret.

36. *Pepper, Black* (*Class IV*).—0.125 : 100 = F; 0.1 : 100 = S.

The freshly powdered drug was infused (0.5 per cent.), with bilein-bicarbonate, filtered and diluted with water.

0.5 per cent. = dead and diffluent in 2½ hours.

0.25 per cent. = normal in one hour; dead and flaccid next day.

0.125 per cent. = nearly dead in 3½ hours; dead and flaccid next day.

0.1 per cent. = after a day, very much depressed and flaccid, but alive.

Pepper is another instance of the fair anhtelmintic value of the "sharp" condiments. It will be discussed further under synergistics.

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15. Warren, L. E.: Reports of Chem. Lab. A. M. A., **10**: 60.

37. *Physostigmin.*—0.0006 per cent. = S.

This was not tried for toxicity, but for physiologic effects. The results were quite curious. When placed in the 0.0006 per cent. solution, there was some immediate agitation. On the next two days, the worms were found tightly curled in s form, and unable to straighten out.

38. *Picrotoxin.*—0.01 per cent. = S.

A 0.01 per cent. solution was made in bilein-bicarbonate. The worms appeared normal in an hour. After a day, they were much more depressed, clubshaped, but alive.

Schroeder[2] found 0.1 per cent. picrotoxin harmless to ascarids in twenty-four hours.

39. *Piperin (Class III).*—0.01 = S.

A 0.01 per cent. solution in bilein-bicarbonate produced depression in an hour. After a day, the worms were flaccid and practically dead.

Piperin doubtless plays an important part in the toxicity of pepper but since its toxicity is only ten times as great, the oil, etc., must be the more important ingredients.

40. *Potassium Cyanid (Class III).*—0.03 : 100 = F; 0.01 : 100 = N.

A pure sample (Schuchardt) was used.

0.1 per cent.: normal in six minutes; slightly depressed in thirty minutes; next day dead and beaded.

0.03 per cent.: somewhat depressed in 2½ hours. After five hours almost paralyzed, but move. After one day, same. Transferred to pure water, but dead when seen on fourth day.

0.01 per cent.: no effect immediately, or after one-fourth hour and after one day. Perhaps rather sluggish.

The low toxicity of cyanid is in harmony with the long life of the worms under stale water. Evidently, their oxidation is low. Ascarids are also resistant. They die after 1¼ hours in 3 per cent. KCN, but the effects are rather those of the alkali.

41. *Pumpkin Seed, Freshly Peeled (Class III).*—0.1 per cent = F; 0.01 : 100 = N.

The specimen used was germinable "Yellow Connecticut Field Pumpkin," bought in May. The seeds were peeled, crushed, and rubbed with 100 or 1,000 parts of water and macerated overnight. They were then used, with or without filtration through paper, as follows:

Unfiltered suspensions:

1 per cent.: normal in one hour, found dead in twenty-one hours.

0.1 per cent.: after twenty-one hours, three are found dead, two just alive.

0.01 per cent.: normal after one day, dead and diffluent in two days.

Filtered solutions:

1 per cent.: normal in one hour, found dead in twenty-one hours.

0.1 per cent.: found dead in twenty-one hours.

Old pumpkin seeds: 2:100, S in one day.

A museum specimen of uncertain age (but at least five years old) was used.

Ten gm. yielded 7.3 gm. of shelled seed.

Two per cent. of shelled seed was crushed and macerated in water for one day, and filtered. After one day, the worms were greatly depressed, but alive.

The experiments vindicate the old and somewhat discredited confidence in this cheap and highly efficient, and presumably harmless anthelmintic. It should be reëxamined clinically. Inefficient results may have been due to the use of aged drug, which is shown to lose greatly in activity. The subject is further discussed under squash seed.

42. *Quassia Extract* (*Class IV*).—0.5 per cent.: 100 = S for one day; 0.05 : 100 = N for one day.

The official quassia extract was dissolved in water. With 0.5 per cent. the worms showed very slight depression in ten minutes, and in two hours. After a day, they were alive, but exhibited very slow conduction; only a part of the body contracting.

With 0.05 per cent. no effect was observable in a day.

Quassia was tried because of its use in enemias against oxyuris. Apparently it is a very weak anthelmintic, as are also all other simple bitters that were tried (gentian, picrotoxin, quinin, and strychnin).

43. *Quinin Hydrobromid* (*Class III*).—0.1 : 100 = F in three hours; 0.02 : 100 = N for one day.

0.1 per cent. produced immediately considerable agitation. When inspected after three hours, the worms were dead.

0.02 per cent. caused very slight agitation immediately. In fifteen minutes, the animals appeared normal; also on the next day.

When a trituration of quinin and chalk is placed at the bottom of a conical glass of water, the worms tend to draw away from it.

The toxicity of quinin is rather low, considernig its toxicity to other lower animals. The apparently sluggish metabolism of the worms may furnish an explanation. Schroeder[2] found ascarids even more resistant; they appeared normal after four hours in quinin hydrochlorid.

44. *Saccharin Sodium (Class V)*.—1 : 100 = S.

Saccharin was dissolved by addition of one-half its weight of sodium bicarbonate. The tests were made in May:

1 per cent. of saccharin with ½ per cent. of bicarbonate: after one day, two worms are almost dead. Five others are depressed, but quite alive.

0.2 per cent. of saccharin with 0.1 per cent. of bicarbonate: apparently normal in one day.

Saccharin is practically not anthelmintic, although it is not quite indifferent.

45. *Saccharose*.—Indifferent. In 1 per cent. the worms behave normally at the end of a day. When placed on solid granulated sugar, they burrow into it, in very striking contrast to their violent withdrawal from crystallized sodium chlorid. The worms therefore are rather unsusceptible to osmotic irritation.

46. *Santonin in Bilein Bicarbonate (Class II)*.—0.01 : 100 = F in one day; 0.001 : 100 = N in one day.

Santonin is very sparingly soluble in water. Several solvents were tried. The best results were obtained with the bilein-bicarbonate mixture, which was intended to imitate the intestinal fluid.

Santonin-Bilein Bicarbonate.—The stock mixture was made with santonin, 0.1; bilein, 0.04; sodium bicarbonate, 1; water, 100. The mixture is turbid, and was shaken before using. The dilutions were made with water. When nine parts of water are added to one part of the stock suspension, solution becomes almost complete. The following concentrations are stated as santonin, the other constituents being in the proportions just explained.

0.1 per cent.: immediate moderate agitation. After end of a day, one worm is just alive. The other four are dead and diffluent.

0.01 per cent.: in one hour, the worms are somewhat depressed and are twisted in a ball. After one day, they are found dead.

0.001 per cent.: after one day, the animals are found alive, but curled and depressed.

Santonin Bicarbonate.—A mixture of santonin, 0.1 gm.; sodium bicarbonate, 1 gm., and water, 100 c.c. Considerable remains undissolved, but is left in the mixture. Worms placed in the suspension (0.1 per cent. santonin) immediately show agitation. After a day, they are found dead, flaccid and nodular.

Santonin Bilein.—Santonin, 1 gm.; bilein, 1 gm., and water, 100 c.c., gives a turbid suspension. This is still slightly cloudy when one part of the suspension is diluted with nineteen parts of water. The following experiments were tried:

Santonin and bilein, each 0.05 per cent.: immediately, fair agitation; 1½ hours, relaxed, almost dead; one day, dead and flaccid.

Santonin and bilein, each 0.005 per cent.: practically normal for one day.

These results show a very high toxicity (Class II in the presence of bilein, which acts presumably as a solvent, but possibly by favoring the penetration of the drug. The relatively poor results of the older observers (Schroeder and Trendelenburg) are probably attributable to the absence of the bile, that is, to incomplete solution. "Sodium santoninate" does not possess this high toxicity.

Hall and Foster[1] found santonin rather disappointing in dogs and not very practical in pigs. The explanation of their poor results is not clear in view of the undoubted clinical efficiency in human subjects. We shall recur later to the mechanism of actions, etc.

47. *Santoninate of Sodium (Class IV).*—0.5 : 100 = F; practically; 0.2 : 100 = N.

Solutions (1 per cent.) are neutral to litmus. The solid salt is strongly repulsive to the worms.

1 per cent.: immediately there is very considerable agitation, and the worms appear rather stiff. In 2½ hours, they are apparently dead, but move feebly. After one day, three were found dead, the other two almost dead, the anterior half of the body apparently stiff, whilst the posterior half can move.

0.5 per cent.: immediately slight agitation, 1¼ hours depressed; one day, relaxed and almost dead, but respond slightly.

0.2 per cent.: no apparent effect for one day.

48. *Saponin (Class III).*—0.1 : 100 = F in one day; 0.01 : 100 = N in one day.

The experiments were made mainly for possible physiologic interest. Merck's "pure saponin" was used.

0.1 per cent.: depressed and sluggish in one hour; after one day, found dead and diffluent.

0.01 per cent.: for one day, somewhat sluggish and curled, but practically normal.

49. *Soap (Class III).*—0.1 : 100 = F within forty-five minutes; 0.03 : 100 = N, practically, for one day.

Powdered castile soap was used.

0.1 per cent. produced immediate agitation. When inspected after forty-five minutes, the worms were dead and slimy.

0.03 per cent. also produced immediately considerable agitation. After ten minutes they were nearly normal, perhaps slightly depressed. No further change had occurred in three hours and in one day.

0.01 per cent. produced practically no effect.

Soap was investigated because of its local use in enemas against oxyuris. It would evidently be highly effective. In view of the rather low toxicity of saponin and of bile salts, the high efficiency of soap is evidently not due to lipolysis, but rather to the alkalinity. In fact, the toxicity of soap is about the same as that of sodium carbonate.

50. *Sodium Bicarbonate* (*Class V*).—1 : 100 = N.

Powdered sodium bicarbonate produces repulsion. No effect whatever was noted from a 1 per cent. solution in one day, and from a 0.2 per cent. solution in four days.

The harmlessness of sodium bicarbonate in concentrations of 1 per cent. and less renders it a suitable solvent for difficultly soluble acids, such as saccharin.

51. *Sodium Carbonate Crystals* (*Class III*).—0.1 : 100 = F within thirty-five minutes; 0.03 : 100 = S for one day.

The undissolved crystals are strongly repulsive to the worms. 1 per cent.: within eighteen minutes the worms are dead and slimy.

0.1 per cent.: immediate slight agitation. In thirty-five minutes, the worms are found dead.

0.03 per cent.: immediate slight agitation. In 2½ hours, they appear normal. In one day, they are weak, but alive.

0.01 per cent.: no visible effect within a day.

Sodium carbonate is therefore a quite strong anthelmintic, provided that it comes in direct contact with the worms.

Crozier[16] has investigated the sensory reaction of earthworms to alkalies in detail. He found that NH_4OH is much more active than equimolecular NaOH; presumably because of easier penetration.

Ascarids have a relatively high resistance to sodium carbonate, 5.8 per cent. requires five hours to be fatal (Schroeder[2]). This is presumably due to the resistance of the chitinous envelope. Schroeder found sodium hydroxid 0.2 per cent. fatal in twenty minutes, whilst 0.022 per cent. produced no effect.

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[16] Crozier, W. J.: Am. J. Physiol., **45**: 315, 1918.

52. *Sodium Chlorid* (*Class V*).—1 per cent. = N for one day.

Dry crystals and presumably strong solutions cause repulsion. A 1 per cent. solution had no effect in one day.

53. *Spigelia* (*Class IV*).—0.5 : 100 = F; 0.1 : 100 = nearly F; 0.02 : 100 = N.

An active specimen was obtained from Kraemer, and used as filtered infusions in May.

In 0.5 per cent., the worms were practically dead in two hours. When inspected in twenty-five hours, they were dead.

In 0.1 per cent., they were just alive in twenty-one hours.

In 0.2 per cent., they remained normal during two days.

An old museum specimen gave nearly identical results:

In 0.5 per cent., the worms were almost dead in two hours, but retained a good tone. In one day, they were all dead, the tails filiform.

In 0.1 per cent., they remained normal during the day.

These results indicate that spigelia has a demonstrable anthelmintic action, but so weak that it would be quite unreliable. This agrees well with the modern clinical estimate. Hall and Foster[1] found it practically inefficient in dogs.

54. *Squash Seed, "Hubbard," Fresh Peeled* (*Class III*).—0.1 : 100 = F in one day; 0.01 : 100 = F in one day.

These experiments were made on germinable seed, purchased in a seed store and tested in May. The peeled seeds were crushed, rubbed with twenty parts of water, macerated overnight, and then diluted, with or without filtration. Exactly the same results were obtained, so that the active constituents are not removed by filtration. The results were as follows:

1 per cent.: normal in one hour, found dead in twenty-one hours.

0.1 per cent.: found dead in twenty hours.

0.01 per cent.: normal for two days.

Effect of Boiling.—This was investigated in an earlier series. The seed was taken directly from the squash. Thirty gm. of the fresh seed yielded 10.25 gm. of shelled seed. The latter was used as a macerate, filtered with and without previous boiling.

Unboiled Filtrate	Boiled Filtrate
0.4 per cent. = F in 1 day	2 per cent. = F, practically in 2 days (3 dead, 2 almost dead)
0.08 per cent. = N for 1 day	1 per cent. = N, practically for 1 day
	0.4 per cent. = N for 1 day

Squash seed like pumpkin seed, is evidently a quite powerful and harmless anthelmintic. The active constituents appear to be soluble in water, and could be administered as solutions or as an unfiltered "milk." They are gradually destroyed by boiling. They probably also diminish with the aging of the seeds and this may be responsible for the unreliable results that seem to have been sometimes obtained.

The unlimited supply, the low cost and the harmlessness of the pumpkin and squash seeds should revive clinical interest, especially at this time when other active anthelmintics are scarce.

55. *Strychnin Sulphate.*—0.01 : 100 = N.

This was used for its physiological interest. In the 0.01 per cent. solution, the worms became very lively, and curled up, similar to physostigmin, but not as much. They were observed in one-half hour and one day. Schroeder[2] found that 0.5 per cent. solution of strychnin salt left ascarids normal over three hours.

56. *Tannin* (*Class III*).—0.05 : 100 = F in one day; 0.01 : 100 = N for one day.

Solid tannin produces immediately strong repulsion. All the solutions also cause immediate agitation.

0.1 per cent.: normal in an hour; dead and stiff in a day. The worms killed by this and the following solution show a curious protrusion of the mouth.

0.05 per cent.: similar to the preceding.

0.01 per cent.: except for the immediate agitation, the worms show no effect for two days.

Tannin is a fairly strong anthelmintic, which may be quite useful for enemas. It would, of course, be useless by mouth.

57. *Thymol* (*Class II*).—In BB., 0.01 : 100 = F; 0.002 : 100 = S; 0.0005 : 100 = N; 1/10 saturated solution = F; 1/30 saturated solution = N.

Thymol was tried by two methods, that is, as dilutions of a saturated solution with the aid of chalk trituration, and by trituration (0.01 per cent.) with the bilein-bicarbonate mixture. All the solutions produced immediately considerable agitation.

The chalk-trituration gave the following results:

Saturated solution: fatal within three-fourths hour.

One-tenth saturated solution: in one hour, very flabby, but move. After one day, dead;

One-fiftieth saturated solution: normal in one and one-half hours and one day.

The bilein-bicarbonate solutions gave the following:

0.01 per cent.: practically dead in 2½ hours;

0.002 per cent.: still somewhat agitated after one hour. After one day, practically dead;

0.0005 per cent.: considerably depressed and sluggish but not fatal in one day.

The results show that thymol is a very active vermicide, in agreement with the clinical experience. Hall and Foster[1] found it of rather variable efficiency in dogs.

58. *Turpentine Oil (Class II?)*.—$\frac{1}{10}$ saturated solution = F in one day; $\frac{1}{30}$ saturated solution = N for one day.

This was used only as dilutions of a saturated solution made with the aid of chalk.

The one-tenth saturated solution produced immediately considerable agitation, lasting over an hour. Next day, four worms were dead, flaccid and beaded. One was alive.

The one-thirtieth saturated solution produced no marked effects during a day.

Turpentine is thus a very powerful anthelmintic; but its toxic actions interfere with the use of efficient doses. This was also the conclusion of Hall and Foster.[1]

Tyramin.—0.02 : 100 = N for one day.

This was tried for its possible physiologic interest. The above concentration caused slight temporary agitation, but no other effect was observable for a day.

COMBINATIONS OF ANTHELMINTICS

The usual preparatory treatment with spices gives the opportunity of combining their toxicity with that of the more active anthelmintics. The use of pumpkin seed as an adjuvant offers similar opportunities. The active anthelmintics are not often combined in modern practice. Such combinations would be desirable, however, should it be found that the toxicity to the worms is additive; for the toxicity to the mammalian host is often based on different actions, so that it would not be additive. These considerations prompted the following experiments. All were made with filtered bilein-bicarbonate solutions.

Stock Solutions.—These were mixtures of equal parts of the just fatal concentrations of both drugs. They would therefore contain one-half of the fatal concentration of each constituent.

The stock solution should be just fatal if there were simple summation of the toxicity of both drugs; subfatal if there were only partial summation; and superfatal (that is, capable of dilution) if there were potentiation.

The results will be presented in this form, that is, as the toxicity of the stock mixtures, containing 50 per cent. of the fatal concentration of each of the two drugs.

60. *Pepper and Aspidium Oleoresin.*—Fatal concentration = pepper, 0.125 per cent.; aspidium oleoresin (III), 0.002 per cent.

Stock solution = pepper, 0.0625 per cent.; aspidium oleoresin (III), 0.001 per cent.

Theoretical toxicity = 100.

Results: stock solution = F; one-half stock solution = F; one-sixth stock solution = N, practically.

Since a one-half stock solution is fatal, the toxicity is at least twice the summed toxicity of the ingredients. There is, consequently, actual summation.

61. *Pepper and Chenopodium Oil.*—Stock solution = pepper, 0.0625 per cent.; chenopodium oil, 0.005 per cent.

Results: Stock solutions = F; one-half stock solution = F; one-sixth stock stock solution = S (much depressed, but alive).

Again, as in No. 60, there has been a potentation of at least 100 per cent.

62. *Pepper and Thymol.*—Stock solution = pepper, 0.0625 per cent.; thymol, 0.001 per cent.

Results: Stock solution = F; one-half stock solution = F; one-sixth stock solution = S, much depressed but alive.

As with aspidium and chenopodium, the pepper has not only summed, but more than doubled the toxicity.

63. *Pepper and Santonin.*—Stock solution = pepper, 0.0625 per cent.; santonin, 0.001 per cent.

Result of stock solution = F. There is at least full summation; the problem of potentation was not investigated.

64. *Chenopodium and Thymol.*—Stock solution = chenopodium oil, 0.005 per cent.; thymol, 0.001 per cent.

Result of stock solution = F just; four worms are dead, one barely alive. Consequently, there was just summation.

65. *Chenopodium and Aspidium.*—Stock solution = chenopodium oil, 0.005 per cent.; aspidium oleoresin (III), 0.001 per cent.

Result of stock solution = just F, that is, practically dead. This corresponds to simple summation.

66. *Thymol and Aspidium.*—Stock solution = thymol, 0.001; aspidium oleoresin (III), 0.001.

Result of stock solution = just fatal; that is, practically dead. This again corresponds to simple summation.

67. *Chenopodium and Santonin.*—Stock solution = chenopodium oil, 0.005 per cent.; santonin 0.001 per cent.

Results of the stock solution = just F., that is, practically dead. This is simple summation.

68. *Aspidium and Santonin.*—Stock solution = aspidium oleoresin (III), 0.001 per cent.; santonin, 0.001 per cent.

Result of the stock solution = F, that is, full summation.

69. *Thymol and Santonin.*—Stock solution = thymol, 0.01 per cent.; santonin, 0.001 per cent.

Result of stock solution = F, that is, full summation.

The combination of the active anthelmintics (aspidium, chenopodium, santonin and thymol) with each other gives full summation. The combinations of any two of these permits the dose of each to be reduced to one-half. Combination with pepper gives not only complete summation, but actual potentation of the effects.

"REPULSIVE EFFECTS" OF ANTHELMINTICS

The apparent discrepancy between the low experimental vermicide efficiency and the high clinical efficiency of santonin led to various attempts to explain its effects by a "vermifuge" action. The present investigation has removed the need for these rather forced explanations in that it has shown that santonin is highly toxic to worms in the presence of a suitable solvent, that is, bile salts. However, the experiments on "repulsive" effects are still interesting, since repulsive effects doubtless do occur, and may have more or less to do with the clinical efficiency; although they are not at all specific for santonin, but are produced by practically all vermicides.

Schroeder[2] observed that ascarids are put into much more active movement by santonin. This he interprets as a sign of discomfort, which drives the worms into the colon, from which they are then expelled by the cathartic, in a living condition.

These active movements apparently correspond to the "agitation" observed in earthworms when they are first placed in almost any toxic solution Trendelenburg[5] showed that the stimulant effect occurs also in ganglion-free preparations of the earthworm, and considers that this effect is at least quantitatively characteristic for santonin. However, it is also given by chenopodium oil and evidently also by other poisons.

Schultz[17] also believes that anthelmintics produce "rapid vermiform movements" in the parasites; if their irritation is sufficiently great the worms will attempt to escape. If the concentration rises above the stimulant concentration, the worms become paralyzed.

The irritant, agitative, "repulsive" and "escape" movements can be excellently observed in the simplest manner on earthworms. They are evidently of the same type as those of the parasitic worms, and much more easily studied and understood. Several arrangements are suitable for these observations.

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[17] Schultz, W. H.: J. A. M. A., **57**:1102, 1911.

Agitative Effect.—When worms are placed in water in a conical glass, they generally assume a vertical position, head down, and body waving gently. When an irritant is added, the movements become much more violent, or even whiplike. Doubtless worms in this state of incoordinated movement would be unable to resist the peristaltic sweep.

Repulsive Effect in Conical Glasses.—If the irritant is introduced in the bottom of the glass, it comes in contact with only a part of the worm (usually the head). This is promptly withdrawn or snapped back into the unpoisoned stratum. The worms may thus "withdraw" a small distance from the irritant; where it comes to rest. This occurs with sodium santoninate but equally well with sodium chlorid—in fact, with any irritant. In the intestines, it would tend to drive the worms down the intestines a short distance ahead of the irritant.

Repulsive Effects Studied on Leaf Mold.—This natural habitat of the worms is also suitable to illustrate their "withdrawal" or "repulsion" by irritation or toxic substances. It is easy so to arrange the leaf mold that parts of numerous worms are exposed, practically at rest. These are sprinkled with a little of the dry substance or solution. The following observations were made:

Powdered chalk produces only a momentary, mechanical reaction. Chalk saturated with aspidium or chenopodium causes immediate extensive withdrawal, and violent whiplike movements. Dry bilein also produces a whip reaction.

Kousso, 2.5 per cent. solution gives immediate reaction from the head, apparently less from the tail. Quassia, ½ per cent.; solution of solid extract gives no reaction. Fresh onion prompts immediate escape.

Dry *santonin, sodium chlorid* and *sodium bicarbonate* cause immediate withdrawal. Dry *saccharose* gives only a momentary mechanical reaction which stops at once.

Repulsive Effect on Blotting Paper.—When the worms are crawling over the paper, a drop of the reagent is placed in their path:

Bilein-bicarbonate has no effect.

Aspidium (0.03 per cent. in BB) causes deflection of the course.

Pepper (0.125 per cent. in BB) causes deflection and more energetic movement.

Sodium santoninate or *mercuric chlorid* causes the worm to jerk back at once.

Glass Spiral Experiment.—This experiment was invented by Straub (Fuehner,[12] p. 44) to show the repulsive effect of santonin on ascarids. The intestines are supposed to be represented by a glass spiral, filled with warm Bunge's fluid. The ascarids tend to settle to the bottom of the tube. A strong solution of sodium santoninate is then introduced near the worms, and they generally move away as the santonin diffuses into the solution.

The same phenomena can be demonstrated with earthworms placed in such a spiral filled with water. When a crystal of sodium santoninate is introduced near to the worms, their movements become more lively, and generally carry them some distance away; but as the santoninate becomes diluted by diffusion, the worms tend to gravitate to their old position.

Again, the phenomenon is not at all characteristic of santonin, but is shared by any irritant, and can be demonstrated much more strikingly by adding a few drops of 1 per cent. mercuric chlorid. With this, the worms will not only withdraw, but they will be killed and coagulated a little distance (for instance, two inches) from their original position.

Zigzag Tube.—In the glass-spiral experiment of Straub, the movements of the worms are complicated by the effects of gravity. It was sought to avoid this by using a zigzag tube, about 1 cm. internal diameter, with eight bends of about 45 degrees about every 10 cm.; the ends being bent vertically upward. The tube was filled with water, and a few worms were introduced. It was hoped in this way that even minor repulsions by substances introduced at one end would be indicated by the distribution of the worms after a considerable time, say twenty-four hours. It was found, however, that the normal wanderings of the worms were rather capricious, so that more reliance was placed on the immediate reactions. The average distance of unpoisoned worms from the starting point was as follows:

	20 Minutes	40 Minutes	3 Hours	6 Hours	24 Hours
Experiment 1	....	3.5	10	20	28
Experiment 2	7.5	12.0	..	..	..
Experiment 3	14.0	....	40	33	33
Average	11.0	8.0	25	27	30

Santonin.—This had a distinct repellant action when placed in a solvent medium (bilein-bicarbonate). In water, the effect is almost nil. The following is perhaps the most instructive experiment:

A fair sized worm is placed in the tube filled with bilein-bicarbonate solution. It is slightly restless. Some santonin crystals are added. The worm jerks back on touching the crystals, some of which adhere to the mucous coating of the skin. The worm then wanders promptly to the other end of the tube, the crystals still adhering. A few crystals of santonin are now added to this end, the worm tries to come to the surface of the water, but in doing so must pass the santonin crystals. This causes him to sink back immediately. This maneuver is repeated several times.

Another worm is added and this also shows the same phenomena.

Santonin-Adhesion Phenomenon.—Attention was called to the adhesion of the santonin crystals to the skin of the worm by mucous threads. Chalk or cane sugar failed to show this adhesion. Santonin shows it only in the presence of sodium bicarbonate; it adhered in 1 per cent. sodium bicarbonate, but did not in 0.04 per cent. bilein without bicarbonate.

Aspidium Oleoresin.—This is also markedly repellent. A worm is placed in the tube filled with water, and some aspidium-chalk powder is added; the worm draws back sharply when its head comes within 5 cm. of the powder. Gradually it withdraws beyond the first bend, occasionally attempts to come back, but always turns around and withdraws when it comes within 5 to 7 cm. of the powder.

Another tube is filled with water, five worms added, some aspidium chalk sifted in, and the average position of the worms observed. This is contrasted with the normal averages as follows:

AVERAGE MIGRATION OF WORMS

Time	In Water Alone, Cm.	With Asphidium, Cm.
40 minutes	8	10
3 hours	25	18
24 hours	30	65 (worms lively)

Pelletierin Tannate.—This is also actively repellent. The tube is filled with water, a worm placed in one end, and a little pelletierin tannate powder is added, forming a small lump. The worm jerks back sharply whenever its head touches the tannate, then turns around and creeps away. It often tries to come back, but always turns away as soon as it comes within about 5 cm. of the pelletierin. Finally, the worm settles down into a ball, at about this distance.

Granatum.—This acts similar to the pelletierin. It was tested by the average migration of the worms.

Time	Average Normal Migration, Cm.	Average Migration after Adding 2 C.c. of 2 per Cent. Infusion of Granatum, Cm.
20 minutes	11	18
40 minutes	8	20 (in tight wad)
1½ hours	..	Same

Chenopodium Oil.—This repels like aspidium. When added on chalk, the worm withdraws rapidly to the bend of the tube, and does not approach closer than 7 or 8 cm.

Black Pepper.—Repulsion; the worm withdraws beyond the next bend. It wanders back and forth, but keeps at least 3 cm. away from the powder.

Onion.—Repulsive. A small piece of onion scale repels the worm and keeps it about 2 cm. away.

Mustard Oil.—Strongly repulsive. A little of a 0.5 per cent. solution in 50 per cent. alcohol is added: the worm jerks back violently at every approach.

Squash Seed.—A crushed seed is added. The worm jerks away and escapes. It returns and burrows rapidly into the emulsion; but is evidently very uneasy, turns back, and escapes again.

Copper Sulphate.—A little of a 0.1 per cent. solution is introduced. The worm jerks away sharply on its approach and crawls away. It approaches again, but again jerks away and makes for the other end of the tube.

Tannin.—This makes the worm uneasy and causes withdrawal.

Quassia Chips.—These apparently have little if any effect on the movements of the worm.

Bilein.—2 c.c. of 1 per cent. added to the upright tube. This causes slight agitation whenever the worm happens to go through the bilein stratum, but it makes no effort to escape.

Hydrochloric Acid.—Some 1 per cent. acid is added, causing violent agitation and death before the worms escape.

Saccharose Crystals.—These have no effects.

Sodium Chlorid Crystals.—The worm jerks back immediately when it is within 2 cm. of the crystals, and then crawls away to the opposite end of the tube.

SUMMARY

All clinical anthelmintics are markedly toxic to earthworms. This simple test may therefore be used for determining whether a given substance has any anthelmintic properties. It may also be used to determine the relative activity of different samples of a given drug. It could not be used to compare the clinical value of different anthelmintics, since this often involves factors other than simple vermicidal efficiency; such, for instance, as absorption, local and general toxicity, etc. For instance, the highest vermicidal efficiency is possessed by substitutes that are not clinically available for this purpose; namely, mercuric chlorid, cupric sulphate, and mustard oil.

Aspidium, chenopodium, pelletierin, thymol, betanaphthol, and chloroform are highly effective. So is santonin in the presence of an appropriate solvent (bile-salts and sodium bicarbonate, simulating the intestinal fluid).

Somewhat less effective, but still quite toxic are kamala, kousso, and granatum. Spigelia is rather feeble.

Fresh (germinable) pumpkin seed and squash seed are quite highly efficient, the active principle being soluble in water and destroyed by boiling. In view of their cheapness, availability, and presumably low toxicity to men, renewed clinical interest in these is indicated.

Spices and "sharp" substances including mustard, pepper, onion and cantharidin are quite toxic. Their use in the preparatory treatment is therefore well justified (except of course cantharidin). Indeed, pepper potentiates or synergizes the effects of the more active anthelmintics.

Mixtures of the active anthelmintics give simple summation of efficiency. This may be useful for decreasing their toxic effects on the hosts. It would need to be determined by further animal experiments and clinical trial.

Oleoresin of aspidium appears to be quite stable, although the dry rhizome deteriorates. Different samples agree fairly in activity. The deposit that occurs in some oleoresins appears to have little if any influence in the activity.

Different samples of pelletierin are also of fairly uniform activity. The "pelletierine tanret" is a secret preparation without any advantage.

Most substances that are toxic to earthworms produce a primary irritation or agitation, that results in the withdrawal of the worm from the neighborhood of the poison. This is observed with santonin, but is no more marned with this than with other anthelmintics. By virtue of this effect, anthelmintics doubtless often "expel" the parasite when the concentration does not rise sufficiently high to kill the worm.

THE CHEMISTRY OF DIPHTHERIA ANTITOXIN—A REVIEW[1]

ALBERT C. CRAWFORD AND MARJORIE G. FOSTER

(From the American Journal of Pharmacy, November, 1918, p. 766)

The indications at present are that rational therapeutics will advance mainly by using chemotherapeutic methods, or by adopting those used by nature, that is, by the use of the products of various glands and of antitoxins, etc. With this latter idea in view, we have endeavored to present the results of investigations on the chemical nature of the diphtheria antitoxin, and hope to follow this summary with a report of our own experiments.

Up to the present time, very little has been ascertained as to the chemical nature of the antitoxins. Weichardt says that in spite of hundredfold attempts to isolate the diphtheria antitoxin, as yet these have been futile.[2]

Reports differ as to the response of diphtheria antitoxin to reagents. Since the antitoxin has never been isolated, the colloids of the serum presumably may modify the reaction in various ways, and protect the antitoxin from their action. Normally the degree of concentration of colloids in the serum offers an obstacle to the activity of proteolytic ferments.[3] It may be that in a pure form diphtheria antitoxin might thus react differently than when in serum, in milk, or if combined with its toxin. Another explanation may be that the antitoxin is a colloid, at least under ordinary conditions, with a large molecule, and may contain numerous reactive groups. In colloids the properties due to chemical nature may be overshadowed by their physicochemical condition.

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[1] From the Division of Pharmacology, Stanford University Medical School, San Francisco, Cal. This report was supported by a grant from the Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association.

[2] Abderhalden, E.: Biochem. Handlexikon, **5**: 518.

[3] Bronfenbrenner, J.: Proc. Soc. Exper. Biol., **13**: 19, 1915.

During immunization, lowering of the freezing point and of the osmotic pressure of the serum occurs. The electrical conductivity decreases in proportion to the antitoxic acivity.[4]

According to Mellanby, a constant current (100 volts), if passed through a slightly acidified antitoxic serum by means of zinc electrodes, causes precipitation. The protein mass collected at the anode, when dissolved in weak NaOH, possesses antitoxic value. The serum also possesses antitoxic powers.[5] Precipitation by zinc ions may here play a part. But Field and Teague, using platinum electrodes, claim that both diphtheria toxin and its antitoxin are electro-positive and pass to the cathode from passage of the 110-volt direct current.[6] They claim the response is the same whether alkaline or acid solutions are used. However, the technic of these experiments of Field and Teague has been criticised by Landsteiner and Pauli[7] and by Bechhold.[8]

It is well to remember that certain proteins in neutral solution do not move toward either pole, but in acid solution travel toward the cathode, and in alkaline solution toward the anode.[9] An amphoteric electric character, which is important in maintaining the reaction of colloids, is better preserved in a large than in a small molecule.[10] As yet no one has succeeded in isolating antitoxin by means of the electric current.

Direct sunlight is injurious to antitoxin.[11] Short exposure, that is, several days, does not seem to materially injure it.[12] Long exposure (four months) to day-light decreases its activity.[13] The injurious action of yellow and of red light is slight (six months' exposure); that of blue light is somewhat more marked (five months); while green light does not affect it on three months' exposure, but after six months causes marked injury.[14] According to Müller, O, H, N and CO_2 injure antitoxin. The standard unit of diphtheria antitoxin in Germany is kept over P_2O_5 in vacuo and in the dark.

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[4] v. Szonagh, F., and Wellmann, O.: Deutsch. med. Wchnschr., **24**: 421; Butjagin, P. W.: Hyg. Rundsch., **12**: 1209, 1902.

[5] Mellanby, J.: Proc. Roy. Soc. London, Series B, **80**: 399, 1908.

[6] Field, C. W., and Teague, O.: J. Exper. Med., **9**: 86, 1907.

[7] Landsteiner, K., and Pauli, W.: Wien. med. Wchnschr., **58**: 1010, 1908.

[8] Bechhold, H.: München. med. Wchnschr., **54**: 1921, 1907.

[9] Hardy, N. B.: J. Physiol., **24**: 288, 1899; Pauli, W.: Beitr. Z. physiol. u. path. Chem., **7**: 531, 1905-6; see also Bancroft, W. D.: J. Phys. Chem., **19**: 349, 1915.

[10] Pick, E. P.: "Biochemie der Antigene." In W. Kolle and A. von Wassermann, "Handbuch der Microorganismen, **1**: 703, 2 Ed.

[11] Palmirski, L., and Orlowski, W.: reference in Centralbl. f. Baktr., **9**: 916, 1896.

[12] Marenghi, G.: Centralbl. f. Bakt., **22**: 520, 1897.

The experiments on dialysis with those on ultrafiltration and on diffusion of the antitoxin, open up the question of the size of its molecules or particles. Antitoxin, at least under the condition as we know it, does not dialyze through parchment paper.[15] We have no data as to whether certain membranes react with antitoxins or absorb them.[16]

There is a variable loss of antitoxin when drawn through a Berkefeld or Chamberland filter. According to Dziergowski, it passes through uninjured.[17] Diphtheria antitoxin does not pass through a Chamberland filter, if the pores are closed by gelatin and the fluid is not forced.[18] It is held back by gelatin filters.[19]

Diphtheria antitoxin is retained by collodion filters.[20] The diffusion constant of diphtheria antitoxin against gelatin at +6° C. is 0.0015, that of the toxin is 0.014, and of NaCl is 0.94,[21] and hence is a colloid, at least in the form we meet it, or so intimately associated with colloids as to react with them. Taylor believes it has more colloidal properties than its toxin, but no conclusions regarding molecular weight by the use of Thovert's relation are permissible.[22]

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[13] Müller, F.: Centralbl. f. Bakt., **24**: 316, 1898.

[14] Müller: Centralbl. f. Bakt., **24**: 251 and 316.

[15] For methods see Gibson, R. B.: J. Biol. Chem., **1**: 161, 1905; Pick, E. P., in Krause, R., and Levaditi, C.: "Handbuch d. Immunitatsforschung," **1**: 534; Seng, W.: Zeitsch. f. Hyg., **31**: 516, 1899; Welker, W. H.: Biochem. Bull., **2**: 70, 1912-1913.

[16] See Tschirch, A.: Arch. d. Pharm., **252**: 537, 1914

[17] Dziergowski, J.: Centralbl. f. Bakt., **21**: 333, 1897; Di Martini, L.: Centralbl. f. Bakt., **24**: 360, 1898.

[18] Corbett, L.: Centralbl. f. Bakt., **24**: 386, 415, 1898.

[19] Brodie, T. G.: J. Path., **4**: 464, 1897.

[20] Goucher, L.: Bull. Sc. Pharm., **19**: 129, 1913; also Bechhold: Biochem. Ztschr., **6**: 401, 1907; Glenny, A. T., and Walpole, G. S.: Biochem. J., **9**: 299, 1915.

[21] Arrhenius, A.: Immuno-chemistry, 1907, p. 25.

[22] Taylor, W. W.: Chemistry of Colloids, p. 309.

According to Zunz, charcoal absorbs as much toxin as antitoxin, but not the combination of the two. Proteins interfere with this absorption and $BaSO_4$ absorbs the toxin to a slight extent, but not the antitoxin, nor their combination,[23] while kaolin, argile, talc, kieselguhr, and wood charcoal absorb neither diphtheria toxin, nor its antitoxin, nor their combination. V. Gröer and Kassowitz claim that antitoxin is absorbed by a kieselguhr filter.[24] Aluminum silicate does not absorb the antitoxin, although it absorbs the toxin. This absorptive power is favored by an alkaline reaction.[25]

Silicic acid purified electro-osmotically seems to have a certain capacity for absorption, less for the antitoxin and more for the toxin, than the corresponding acid not purified in this manner (Zunz). Palladium asbestos has been tested on the toxin, but apparently not on the antitoxin.[26] We have no data as to the action of ordinary asbestos on diphtheria antitoxin, but it absorbs 99.9 per cent of its toxin.[27] Various vegetable gums have been found to inactivate the toxin,[28] but these have not been used on the antitoxin.

Direct sunlight with long shaking injures it. This may be due to increased exposure to air (oxydation) or to some mechanical rearrangement of the molecules,[29] but apparently there is no data as to whether shaking in chemically indifferent gases injures it.[30]

Antitoxic serum is insensitive to low temperature. By freezing two or three times, Bujwid kept his anti toxic serum active for over one year.[31] But there is a report that Gorjansky noted a loss in activity from cold.[32] Serum deteriorates six times as fast at 36° C. as when kept on ice.[33]

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[23] Zunz, E.: Arch. Internat. de Physiol., **8**: 227, 1909.

[24] v. Groër, F., and Kassowitz, K.: Ztschr. f. Immunitäts, **22**: 447, 1914.

[25] Zunz, E.: Ztschr. f. Immunitäts. **19**: 326, 1913.

[26] Biltz, W., Much, and Siebert: Beitr. z. exper. Therap. von Behring, 1905.

[27] Wyard, S.: J. Path., **18**:485, 1913-1914; Coplans, M.: ibid., p. 581.

[28] Lindelsheim: Ztschr. f. Hyg., **42**: 308, 1913.

[29] Palmirski, L., and Orlowski, W.: ref. Centralbl. f. Bakt., **19**: 1916.

[30] Wiechowski, W.: Biochem. Ztschr., **81**: 278, 1917.

[31] Bujwid, O.: Centralbl. f. Bakt., **22**: 287, 1897; Ernst, H. C., Coolidge, J. N., and Cook, H. A.: J. Boston M. Soc., **2**: 166, 1897-1898.

The antitoxic serum is said to be slightly weakened by heating to 60-70 C.[34] Mellanby claims it can be heated to 66 C. without injury. The serum retained its antitoxic activity after five hours' heating at 55 C.[35] Partial coagulations of proteins, if it occurs, may carry antitoxin with them, and thus lessen the antitoxic value of the serum. Heating it to boiling quickly destroys the antitoxic value of serum, but dried antitoxic serum will stand heating to 110 C. for one-half hour, and heating to 140 C. for one quarter hour.[36] No doubt the response to heat will depend on the salts present. This has been found to be true in the case of certain toxins, etc. Buchner found that alkali sulphates added to dilute serum increased its resistance to heat 10 C. and raised its activity.[37]

Mellanby found that acetic acid (5 per cent.) and 0.25 per cent. HCl have no deleterious action on antitoxic serum at room temperature, but 0.05 c.c. tenth-normal HCl added to 1 c.c. of antitoxic serum weakened it. Dilute formic acid causes a precipitation from serum.[38] Weak alkalies dissolve certain antitoxic precipitates.[39] According to v. Groër and Kassowitz, dilute alkalies activate the antitoxin, while more concentrated alkalies destroy it. $Ba(OH)_2$ injures antitoxin.[40]

Digestion with 0.2 per cent. HCl and pepsin causes a rapid loss in activity.[41] It is slowly destroyed by tryptic digestion.[42] The addition of NaCl in solution will inhibit pepsin from digesting certain proteins.[43]

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[32] Madsen, T., in Krause, R., and Levaditi, C.: Handbuch d. Immunitätsforschung, **2:** 110.

[33] MacConkey, A.: J. Hyg., **12:** 511, 1912. See also Ito, T.: Zeitschr. f. Immunitäts, **15:** 97.

[34] Van de Velde, H.: Centralbl. f. Bakt., **22:** 534.

[35] Müller: Footnote 14.

[36] Camus, M. L.: Soc. de Biol., **50:** 235, 1898.

[37] Buchner, H.: Arch. f. Hyg., **17:** 138, 1893; Ferrata, A.: Berl. klin. Wchnschr., **44:** 366, 1907.

[38] Brieger, L., and Krause, M.: Berl. klin. Wchnschr., 946, 1907.

[39] Aronson, H.: Berl. klin. Wchnschr., **31:** 453, 1894.

[40] Freund, E., and Sternberg, C.: Zeitschr. f. Hyg., **31**:429, 1899.

[41] Mellanby; also Brieger, L., and Krause, M.: Ztschr. f. Hyg., **31:** 1899.

[42] Mellanby; v. Groër and Kassowitz, p. 449. (See footnote 24.)

[43] Hamburger, W. W.: Arch.: Int. Med. **16:** 341, 508, 1915.

After saturation of the diluted (ten times) serum with CO_2, the precipitate contained only a trace of antitoxin.[44] Brieger and Krause passed CO_2 into a solution of antitoxin containing NaCl, $(NH_4)_2SO_4$ and glycerol, but the precipitate contained no antitoxin, while the filtrate was active.[45]

Chloroform, in 1 per cent. solution, is used as a preservative for antibodies, but causes a precipitate. Diphtheria antitoxin and toxin are very resistant to ether.[46] V. Groër and Kassowitz shook out antitoxic serum with ether and found that the serum retained its activity. This would suggest that the antitoxic agent was not a free lipoid. Antitoxin is not precipitated from serum by 25 per cent. alcohol. Higher percentages precipitate antitoxin according to the protein content.[47] Most of the antitoxin is precipitated by alcohol 35-45 per cent. The action is reversible only to a slight degree. Acetone precipitates antitoxin and long contact inactivates it.[48] Oxalic acid slowly injures it.[49]

Phenol is used in 0.3-0.5 per cent. solution to preserve antitoxic sera. This does not change its antitoxic content.[50] Tricresol (0.0 per cent.) is used to preserve antitoxic serum. Formaldehyde injures it. Putrefaction completely destroys antitoxin.[51]

Aluminium sulphate (5 per cent.) and potassium alum precipitate albumin from the serum, and the filtrate retains its antitoxic value.[52] Dilute NaOH, when added to this filtrate, causes a precipitate which holds the antitoxin. This latter precipitate is soluble in excess of NaOH when more than one third part of potassium alum has been added to the serum.[53]

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[44] Dieudonné: Arb. d. Kais. Ges. Amt. **13**: 293, 1897.

[45] Brieger, L., and Krause M.: Berl. klin. Wchnschr., 1907, p. 946. See also Hiss, P. H., and Atkinson, J. P.: J. Exper. M., **5**: 49, 1900-1901.

[46] Pick, E. P., and Schwarz, O.: Biochem. Ztschr., **17**:491, 1909.

[47] Mellanby: See footnote 5.

[48] v. Groër, F., and Kassowitz, K.: (Footnote 24), p. 448; Brodie, T. G.: J. Path., **4**: 461, 1897.

[49] Brodie: p. 464.

[50] Freund and Sternberg.

[51] Brieger, L.: Festschrift f. R. Koch, 1903, p. 445.

[52] Freund and Sternberg.

[53] See also Razukin, M. A.: J. Russ. Phys. Chem. Soc., **48**: 465, 1916; Razukin, M. A., and Flier, G. D.: ibid., p. 711.

Antibodies can be salted out or floculated by high electrolytic concentration.[54] There are some data that even pure neutral salts on concentration may perhaps injure, or hold back, certain antigens,[55] and this perhaps might occur in the case of antitoxin. Salts with univalent cations, that is, NaCl, Na_2SO_4, $(NH_4)_2SO_4$, NH_4SCN,NH_4Br exercise no influence on the diphtheria-toxin-antitoxin combination in serum.[56]

Antitoxin is not precipitated by NaCl,[56] but NaCl and KCl as used by d'Astros and Rietsch, precipitated the antitoxin incompletely.[57] Likewise, a solution of $CaCl_2$ with NaCl precipitates antitoxin quantitatively from serum.[58] The filtrate after one half saturation with $(NH_4)_2SO_4$ contains no antitoxins,[59] while it is found in the precipitate. Na_2SO_4 (9-12 per cent.) precipitates 50 per cent. of the antitoxin from serum. Na_2SO_4 is used in place of $(NH_4)_2SO_4$ as a precipitant for the antitoxin in serum, partly because $(NH_4)_2SO_4$ is reduced to NH_3 by some albumins. It has about the same precipitating value as $(NH_4)_2SO_4$.[60] Dilute serums react differently with Na_2SO_4 than concentrated ones.

Salts with bivalent cations, that is $Ca(C_2H_3O_2)_2$ 10 per cent. or $MgSO_4$ have no deleterious effect on the antitoxin or its combination with the toxin in serum. The colloids of the serum may perhaps protect the antitoxin from the action of calcium.[61] $MgSO_4$ at 30-37 C. precipitates only 50 per cent. of the antitoxin from serum,[62] but according to Freund and Sternberg the filtrate, after $MgSO_4$ precipitation, contains no antitoxin. These experiments were carried on at 17-18 C.[63] Strontium salts give negative results.[64]

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[54] See London, E. S., and Pakhotina, E. P.: Comp. rend. Soc. de Biol., **80**: 756, 1917.

[55] See Pick, E. P.: "Biochemie der Antigene."

[56] Brieger and Krause: (Footnote 38).

[57] D'Astros, L., and Rietsch, M.: Comp. rend. Soc. de biol., **52**: 337, 1900.

[58] Brieger, L., and Boer: Zeitschr. f. Hyg., **21**: 259.

[59] Freund and Sternberg: (Footnote 40).

[60] Brunner, J., and Pinkus, S. N.: Biochem. Ztschr., **5**: 381, 1907.

[61] Pick and Schwarz: (Footnote 46). See also Osterhout, W. J. U.: Bot. Gaz., **59**: 316, 1915.

[62] Brieger and Boer.

[63] See also Brodie.

[64] Freund and Sternberg.

Zinc salts are said to be especially suited for precipitation of the antitoxin. According to Brieger and Boer, $ZnSO_4$ and $ZnCl_2$ can be used for separating it quantitatively. The Zn precipitate can be dissolved in water by means of weak NaOH (one drop normal solution in 20 c.c. H_2O) and then CO_2 can be used to precipitate the zinc. However by this method there is a question if all of the zinc is removed. In case $ZnSO_4$ has been used, the antitoxin remains in the precipitate, while if $ZnCl_2$ has been used, it goes into the filtrate. Freund and Sternberg obtained no precipitate of antitoxin with $ZnSO_4$ or $ZnCl_2$ after the use of potassium alum and aluminum sulphate even though the solution had been dialyzed eight days. They claim that zinc hydrate precipitates antitoxin. Cobalt salts gave negative results with antitoxin.[65]

The precipitate of iron salts with alkali carries antitoxin mechanically,[66] but it is difficult to free it.[67] The precipitate from the serum by K_4FeCy_6 and $ZnSO_4$, that is, zinc ferro-cyanid, contains antitoxin.[68] Aluminium sulphate and ammonia precipitate about 95 per cent of the antitoxin from serum.[69] We have no published data as to the action of uranyl acetate or of phosphotungstic acid on antitoxin. The former has been used by Brieger in attempts to isolate tetanus toxin.

Brieger and Boer have used heavy metals in their work on the isolation of diphtheria antitoxin. Neutral lead acetate with a trace of NH_4HO precipitates albumin from serum, but the antitoxin remains in the filtrate. This can then be precipitated by shaking with $(NH_4)_2SO_4$, but the results are variable, owing to the solubiliity of the Pb combination. Brieger and Boer believe that antitoxin forms some combination with certain heavy metals and that this combination is soluble in alkalies. $HgCl_2$ will precipitate it if NaCl has been added to the solution.[70]. $CdSO_4$ precipitates 50

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65. Freund and Sternberg.
66. Freund, E., and Sternberg, C.: Ztschr. f. Hyg., **31**: 429, 1899.
67. In this connection, see Brossa, A., and Freundlich, H.: Ztschr. f. Phys. Chem., **89**: 306.
68. Aronson.
69. Brieger and Boer, also Aronson.
70. Brieger and Boer.

per cent. antitoxin, and $CuSO_4$ has also been used as a precipitant.

Platinum chlorid precipitates it.[71]

A solution of copper acetate (1 per cent.) causes a precipitate from antitoxic serum. This when dissolved by means of Na_2CO_3 and treated with CO_2 to remove Cu, is found to have antitoxic properties. However, the filtrate also contains some of the activity.[72]

Nucleohiston precipitates antitoxin.[73]

Nucleoproteins from various tissues possess antigenic properties.[74]

It has been suggested that there was some connection between the specific ferments of the blood and antibodies, but according to Bronfenbrenner—"No definite proof, however, of such an identification has been offered."[75]

Some of the work, which has been done on the chemistry of the diphtheria antitoxin, would suggest that this antitoxin is not a protein, thus Pröscher thought he could obtain an antitoxin free from proteins by digesting these away by means of trypsin,[76] but this work could not be confirmed by Mellanby, Banzhaf,[77] Pick, or Brieger.[78]

Other work would indicate that the antitoxic power of the serum is in relation to the percentage of the proteins, hence a protein. According to Mellanby, heating and freezing both show that the concentration of the antitoxin follows the concentration of the protein on approximately parallel lines.

As Schryver says: "Not sufficient is known yet of the true chemical structure of the proteins to found a rational classification based upon differences of chemical constitution. The classification adopted therefore is more or less of an empirical nature, founded only to a small extent on chemical distinctions, but chiefly on crude differences in physical properties."[79]

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[71] Belfanti. S., and Carbone, T.

[72] Nikanorow, P. J.: in Jahresb. u. d. Fortschn. d. Tier-chemie, **26**: 983, 1897.

[73] Freund, E., Grosz, S., and Jelinck, O.: Centralbl. f. inn. Med., **16**: 913, 937, 1895; Tichomiroff, M.: Ztschr. f. Physiol. Chem., **21**: 90.

[74] Wells, H. G.: J. Biol. Chem., **28**: 11, 1916.

[75] Bronfenbrenner, J.: J. Exper. M., **21**: 222, 1915.

[76] D. R. P. K.: **30**: 13757 (June 21) 1902.

[77] Banzhaf, E. J.: Bull. Johns Hopkins Hosp., **22**: 106, 1911.

[78] Brieger, L.: Festschr. f. R. Koch, 1903, p. 445.

Proteins can be carried out from solution by other colloids, that is, mastic or ferric hydroxid, and upon this property methods have been founded for separating proteins from solution."[80] The reaction is not always reversible, in that the protein cannot always be dissolved out from the precipitate by water. Certain proteins can also be removed from solution by solid substances, which act as absorbents, such as precipitated silicic acid, meerschaum, etc. From certain globulin solutions of about 2 or 3 pro m., the following absorbents take the following percentages:[81]

Meerschaum	100
Precipitated SiO_2	100
Iron oxid	97
Kaolin	64
Serpentin	53
$BaSO_4$	26
$CaSO_4$	18
S	2-3

This absorption is possibly a question of electrochemical affinity.

Proteins can usually be precipitated from solution by means of neutral salts. "If a salt be added to a mixture of proteins, as soon as the limit of concentration is reached at which the more readily precipitated protein falls out precipitation will commence. The precipitate will contain, however, not only the protein of which the salt concentration limit has been reached, but also some of the less rapidly precipitable protein. The only method available, therefore, for separating water-soluble proteins from one another is an imperfect one and this fact accounts for a great deal of uncertainty as to the homogeneity of a large number of proteins which have been submitted to investigation."[82] Proteins, by virtue of their colloidal properties, can "protect" other colloids and prevent the latter from being precipitated from solution.

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79. Schryver, S. B., in Thorpe, E.: Dictionary of Applied Chemistry, **4**: 407.

80. Rona, P., and Michaelis, L.: Biochem. Ztschr., **5**: 365, 1907; **2**: 219, 1906.

81. Landsteiner, K., in Kolle, W., and v. Wassermann, A.: Handbuch d. Mikroorganismen, **2**: Part 1, p. 1246.

82. Schryver: (Footnote 79).

At present there is a tendency to believe that the "proteins fractions of plasma, such as, globulins, etc., are not chemical individuals, but form a series of which the individual members can pass one into another. The more complex proteins are assumed to be liberated from the cells in a state of low aggregation with comparatively small amounts of absorbed simple substances to keep them in a colloidally dispersed state, from which they can be readily flocculated by salts, etc. In the course of time, the state of aggregation is finer and the amount of absorbed substances is increased and the proteins are not then so readily precipitated. By alteration in the state of aggregation and amount of absorbed simpler substances, variations in the physical properties can be produced, and a blood protein can thus be varied in its properties so as to pass from a readily aggregating substance like fibrinogen through a globulin to an albumose."[83]

Diphtheria antitoxin is associated in some way with that division of the proteins which is known as the globulins, and is precipitated from serum by agents which precipitate globulins.[84] Like globulins, some antitoxins are preciptated by heavy metals.[85] However, Mellanby[5] (p. 400) pointed out that the antitoxin did not correspond to the rigid definition of a globulin. The globulins are insoluble in dilute acids, but are soluble in dilute alkali or in dilute neutral salt solution.

According to Starke, globulin in solution is an alkali or acid albumin.[86]

Carbonic acid precipitates globulins from dilute alkaline solution and dissolves them in excess.

The serum globulins are sensitive to reagents and easily denaturize, thus becoming insoluble. Heat, alcohol, and ether quickly precipitate them.[87] They are slowly split by ferments such as pepsin.[88]

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[83] Herzfeld, E., and Klinger, R.: abst. in J. Chem. Soc., **113–114:** 87, Part 1, 1918.

[84] Brodie; Atkinson, J. P.: J. Exper. M., **5:**67, 1900–1901; Belfanti, S., and Carbone, T.: Arch. Sci. Med., **22:** 16, 1898.

[85] Ditthorn and Schultz: Ztschr. f. Immunitäts, **14:** 103, Par. 1, 1912.

[86] See Physiol. Abstr., **3:** 100, 1918.

[87] Tebb, M. C.: J. Physiol., **30:** 25, 1904. See also Chick, H., and Martin, C. J.: J. Physiol., **45:** 61, 1912.

[88] Umber, F.: Ztschr. f. Physiol. Chem., **25:** 258, 1898.

Globulins do not form true salts with metals. The globulins have an acid nature and form combinations with alkalies. Histon, nuclein, neuchleohiston, etc., are said to precipitate globulins.[89]

Acetic acid (0.25 per cent) precipitates diphtheria antitoxin.[90] Acetic acid also precipitates globulins.

Admixed substances may determine the solubility of the globulins, as a globulin preparation soluble in water can become insoluble in water by purification, hence classifications based on solubility must be tentative.[91]

According to Hammarsten, "a sharp line cannot be drawn between the albumins and the globulins, follows from the fact that the albumins can be converted into globulins."[92] The first step in the transformation of albumin into globulin is the formation of pseudoglobulin. This change may be really a question of external properties if the albumin rather than to a true conversion into a globulin, as the resulting substance does not contain glycocoll, which is believed to be present in globulins.

On the addition of colloidal calcium phosphate and kieselguhr to diphtheria antitoxic serum, the ionized salts in the serum cause a precipitate, but the antitoxic value of the serum remains the same, showing that all colloids do not carry down antitoxin.[93]

Immune serum is influenced by salts according to physico-chemical laws of salt albumin combinations.[94] Ammonium sulphate in 50 per cent. saturation, precipitates globulins and antitoxin[95] from serum, leaving the albumin in solution, but precipitation of the albumin occurs when the ammonium sulphate saturation has reached 64 per cent. By this means it can be shown that the antitoxin is not an albumin or necessarily associated with them.[96] The fact that in the precipitation by ammonium sulphate there is no accumulation of antitoxin in any one fraction, would argue against a mechanical precipitation.

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[89] See Osborne, in Abderhalden Biochemisches Handlexikon, **4**: 84.

[90] Gibson, R. B.: J. Biol. Chem. **1**: 169, 1905-1906.

[91] For methods of precipitation, see Samuely, in Abderhalden's Handbuch der Biochem. Arbeitsmethoden, **2**: 360; Morawitz, in Oppenheimer's Handbuch der Biochemie, **2**.

[92] Hammersten, O.: Textbook of Physiological Chemistry, Ed. 7, transl. 1912; 103 refers to Moll-Hoffmeister's "Beiträge," **4** and **7**.

[93] Mellanby: (Footnote 5).

[94] Pick, E. P., and Schwarz, O.: Biochem. Ztsch., **17**: 491, 1909; Pribram, E.: Wien. klin. Wchnschr., **21**: 1079, 1908; Look up Oryng, T., and Pauli, W.: Biochem. Ztschr., **70**: 368, 1915.

[95] Freund and Sternberg.

The response to $(NH_4)_2SO_4$ is different if the serum is first diluted with weak $(NH_4)_2SO_4$ and its $(NH_4)_2SO_4$ concentration then raised to one-half saturation, than when $(NH_4)_2SO_4$ is added directly to undiluted serum.[97]. Highly concentrated sera have different precipitation quality than dilute.[98]

The globulins are usually divided into euglobulins and pseudoglobulins. By precipitation with $(NH_4)_2SO_4$, Freund and Joachim claim to have separated the true globulins into four fractions. By one third saturation, two fractions were obtained—one soluble in water, which was called "euglobulin" and one insoluble in water called "paraeuglobulin." By half-saturation two other fractions were obtained. The one soluble in water was named "pseudoglobulin" and the one insoluble was called "para-pseudoglobulin."[99] The ammonium sulphate separation of euglobulin from pseudoglobulin is an artificial one,[100] and it is still uncertain whether euglobulin and pseudoglobulin are distinct proteins. The amino-acid content of the two fractions is approximately the same. Dale and Hartley find "that a rigidly specific sensitiveness to euglobulin occurs comparatively frequently" but they never succeeded by injection of pseudoglobulin in producing a sensitiveness to pseudoglobulin alone.[101] Chick[101a] suggests that euglobulins are formed by association of pseudoglobulin with a lipoid. Banzhaf believes a separation of the serum globulins by means of solubility in saturated NaCl is as sharp as can be made at present. The euglobulins precipitated from horse serum by saturation with NaCl content contain no antitoxin.[102] According to Dale and Hartley the purification of euglobulin is facilitated by its insolubility in weakly acidulated water.

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[96] Banzhaf, E. J., and Famulener, L. W.: Collected Studies, Bureau of Laboratories, N. Y., **8**: 208, 1914–1915.

[97] Popplewell-Bloxham, W.: Proc. Physiol. Soc., J. Physiol., **26**: 33, 1901.

[98] Brunner, J., and Pinkus, S. N.: Biochem. Ztschr., **5**: 381.

[99] Freund, E., and Joachim, J.: Ztschr. physiol. Chem., **36**: 406, 1912.

[100] Banzhaf and Famulener.

[101] Dale, H. H., and Hartley, P.: Biochem. J., **10**: 429, 1916.

[101a] Chick, H. Biochem. J., **8**: 404, 1914.

Shaking a serum with air is said to produce coagulation and denaturation of the euglobulin, due to alteration in surface energy, leading to absorption of albumin, thus rendering the euglobulins labile; they then coagulate and become insoluble. The process is not due to oxidation. Previous heating and the presence of a hypertonic medium tends to inhibit the effect of shaking, but dilution with H_2O or storage, rendering the euglobulins labile, favors the effect of shaking.[103] Fibrinoglobulin, which belongs to the euglobulin fraction, does not contain the antitoxin.[104]

The globulins of horse serum insoluble in water (precipitating on dialysis) correspond to the globulin fraction precipitated by 28–36 volume per cent. of $(NH_4)_2SO_4$, and those soluble in water correspond to the fraction precipitated by 36–44 volume per cent. $(NH_4)_2SO_4$.[105] According to Ide and Lemaire the antitoxin is all precipitated by from 26–44 per cent. $(NH_4)_2SO_4$.[106]

Only a portion of the globulins seems to contain or carry the antitoxin with it.[107] The coagulation temperature for the "soluble globulins" in immune serum is claimed to be different from that in non-immune;[108] that is, in normal serum it ranges from 65 to 71 C., but in immune serum it begins to precipitate at 71 C. with a maximal coagulation at 75 C. According to Brunner and Pinkus[109] antitoxin of immune serum has not the same precipitation limits as the globulins of normal serum.

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[102] Banzhaf, E. J.: Bull. Johns Hopkins Hosp., **22**: 106, 1911; Banzhaf, E. J., and Gibson, R. B.: J. Biol. Chem., **3**: 253, 1907.

[103] Schmidt, H.: J. Hyg., **14**: 399, 1914

[104] Pick.

[105] See Ficker, M., in Kolle, W., and von Wassermann, A.: Handb. der path. Mikro-organismen, **2**: Part 1.

[106] Ide and Lemaire: Arch. d. internat. d. Pharmacod., **6**: 477.

[107] Belfanti, S., and Carbone, T.: Centralbl. f. Bakt., **23**: 907, 1898.

[108] Seng.

[109] Brunner, J., and Pinkus, S. N.: Biochem. Ztschr., **5**: 381, 1907.

The diphtheria antitoxin globulins have a higher value for cysline nitrogen than have globulins described by Hartley. The histiline nitrogen is lower and the amino nitrogen is slightly higher.[110] According to Seng, the "soluble globulins" of antitoxic serum show an increase in N and C with a diminution in H and possess a higher rotation of the plane of polarization. However globulins of normal and of immune serum have the same freezing point.[111]

Hiss and Atkinson[112] found that the average precipitate with $MgSO_4$ from the sera of immunized horses was more abundant than the average precipitate from the sera of non-immunized horses. This precipitate would contain both the normal globulins of the serum, which have no antitoxic action, and the globulins, which have antitoxic value. The increase in globulin precipitate is not necessarily proportionate to the increase in antitoxic value. Seng found an increase of the so-called "soluble globulins" at the expense of the insoluble ones. According to Joachim,[113] the total protein content of the serum of non-immune animals is only slightly less than in the immune and the globulins increase at the expense of the albumins. These results of Joachim were corroborated to some extent by Langstein and Mayer (1904) who found that in rabbits immunized against typhoid, pneumococcus, streptococcus, etc., the serum globulin rose while the albumin diminished. Joachim believed the increase was mainly in the inactive euglobulin fraction. Others, as Butjagin,[114] also Szontagh and Wellmann[115] claim that the proteins increased during immunization, although the latter workers suggested it might be due to disturbed nutrition. In one horse used by Ledingham the globulin content of the serum showed only a

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110. Banzhaf, E. J., Suguira, K., and Falk, K. G.: Collected Studies, Bureau of Laboratories, New York, **8**: 213, 1914–1915.

111. Hartley, D.: Data on Chemistry of Globulins, Biochem. J., **8**: 541, 1914. See also Rakuzin, M. A.: Optical Activity of Albumins, J. Reiss. Phys. Chem. Soc., **8**: 1251, 1916.

112. Hiss, J. H., and Atkinson, J. P.: J. Exper. M., **5**: 47, 1900–1901.

113. Joachim, J.: Arch. f. d. ges. Physiol., **93**: 593, 1903.

114. Butjagin, P. W.: Hyg. Rundsch., **12**: 1208, 1902.

115. v. Szontagh, F., and Wellmann, O.: Deutsch. med. Wchnschr., 1898, p. 109.

slight change, although the albumin increased, but in this case the serum failed to yield a high grade antitoxin.[116] In a second horse there was a marked increase in globulin content and reduction in albumin along with the increase in antitoxic value. The increase in globulin was mainly in the euglobulin fraction. Ledingham says that the pseudoglobulin and especially the euglobulin fraction are involved in this increase. In the organs, an increase in globulins has not been shown.[117]

It has been urged that perhaps an increase in the globulins of the serum during immunization may be due to changed nutritive conditions; as Githens found an increase in globulins of serum in hungering dogs (1904) and Glässner found that during immunization of rabbits with certain bacterial products a rise in the globulin fraction occurred only if the animal showed severe metabolic changes, but that on cautious immunization this need not appear.[118] The fact that during immunization waxy disease, hepatic hemorrhages, etc., occur would indicate that profound metabolic changes often are produced during immunization.

According to Pick[119] in goat serum the antitoxin is associated with the euglobulin, but in the case of horse serum it is associated with the pseudoglobulins, and while Ledingham found an increase in the globulins in horse serum during immunization, which increase was mainly in the euglobulins, he says that the greater part if not all the antitoxin is associated with the pseudoglobulins, at least when the antitoxin content of the serum is rising. In his experiments with goats he failed to corroborate Pick's finding, that antitoxin was invariably associated with the euglobulins. In the refractory period where abnormal metabolic changes were marked, Ledingham thought it might be associated with the euglobulins. Ledingham found that during immunization of goats the rise in total protein affected mainly the albumin. The recent work of

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[116] Ledingham, J. C. G.: J. Hyg., **7**: 65, 1907.

[117] Pick.

[118] Glaessner, K.: Ztsch. f. exper. Path., **2**: 154, 1905–1906.

[119] Pick: Beitr. d. path. Chem., **1**: 362, 1902.

Banzhaf and Famulener indicates that in goats the unit content and protein increase have no relation. "The unit relationship per gram protein of the pseudoglobulin and euglobulin remained practically the same during the course of the immunization." Banzhaf also found that in horse serum the increase is in the pseudoglobulins, and that the euglobulins contain no antitoxin.[120]

"Pick noted that tetanus antitoxin was precipitated with the pseudoglobulins. However, this association of the antitoxin with one or other globulin is claimed by Pribram to be not always constant, but to vary according to the preservation of the serum, etc. Gibson and Collins have demonstrated the untrustworthiness of any such differentiation of the antibodies as those contained in the euglobulin and those of the pseudoglobulin."[121]

In Hurwitz and Meyer's experiments on immunization, it was found that in rabbits the globulins of the serum only increased when the animals reacted severely, but if the immunization was carried out carefully and with a well-controlled dosage it is not usually accompanied by an increase in the serum globulins, although the immune bodies may attain a high concentration. The rise in globulins occurs long before the animal develops immune bodies in any appreciable concentration.[122] However, it may be remembered that an increase in pseudoglobulins with which the antitoxin is said to be associated might theoretically occur without an increase in total globulins.[123]

Banzhaf believes that when the pseudoglobulins are separated by fractional precipitation with $(NH_4)_2SO_4$, the higher fraction contains more of the antitoxin per gram protein. Acetic acid in small amounts precipitates the pseudoglobulins in saturated sodium chlorid solution.[124]

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[120] Banzhaf and Famulener.

[121] Gibson, R. B., and Collins, K. R.: J. Biol. Chem., **3**: 238.

[122] Meyer, K. F., Hurwitz, S. H., and Taussig, L.: J. Inf. Dis., **22**: 1, 1918; Hurwitz, S. H., and Meyer, K. F.: J. Exp. Med., 1916, p. 515. See also Belfanti and Carbone, Footnote 107.

[123] Schmidt, E. S., and Schmidt, C. L. A.: J. Immunol., **2**: 1916–1917.

[124] Gibson, R. B.: J. Biol. Chem., **1**: 169, 1905–1906.

According to Banzhaf the serum of horses immunized to diphtheria contains 12 per cent. albumin; pseudoglobulin 78 per cent. and euglobulin 10 per cent., while in normal non-immunized serum, albumin is present in 40 per cent., pseudoglobulin in 42 per cent. and euglobulin in 18 per cent.

Moll found that serum heated to 56 for one half hour showed a globulin increase[125] without an increase in alkali albuminate. Heating rabbit serum to 58 C. for one hour, in some cases the euglobulins increased in amount; in other cases the pseudoglobulins increased. In Banzhaf's experiments, on heating horse serum 12 to 15 hours at 57 C. the albumin content fell from 12 per cent. down to 9 per cent.; while the pseudoglobulin fell to 50 per cent. from a normal of 78 per cent., and the normal 10 per cent. of euglobulins increased to 41 per cent. Banzhaf believed the pseudoglobulin was converted to euglobulin. As all the antitoxin, save 7 per cent. lost in heating, is in the pseudoglobulin, the pseudoglobulin is not necessarily antitoxin and Banzhaf argued that a separation of antitoxin and pseudoglobulin could thus be partially affected by heat.[125a] Heinemann claims that pseudoglobulins may be split into fractions, some of which do not contain antitoxin.

By refractometer determinations Homer finds that the pseudoglobulins are not diminished by the Banzhaf method, and claims there is not a conversion of pseudoglobulin into euglobulin, "but rather a more simple physical process involving the agglutination of the particles of the euglobulin already thrown out of emulsoid suspension by the ammonium sulphate. This agglutination permits of easy separation of the euglobulin by filtration through paper."[126] Heat denaturation in alkali sera slowly occurs.

It would seem that a simple method of proving whether the diphtheria antitoxin was a protein or not, would be to find whether it occurred in secretions which contained little or no protein, or a protein which could be easily removed.[127]

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[125] Moll, I.: Beitr. z. Physiol. u. Path. Chem., **4**: 563, 1904; Ztsch. f. exper. Path., **3**: 325, 1906.

[125a] J. Infect. Dis., **19**: 438, 1916.

[126] Homer, A.: Biochem. J., **10**: 292, 1916; **11**: 21, 1917.

Milk of immune animals has been studied. Ammonium sulphate (27–30 per cent.) precipitation from goat's milk gives an antitoxin preparation 400 to 600 times as powerful as the original milk.[128] This precipitate contains 14 per cent. ammonium sulphate, but this method has shown no further results.

According to D'Arsonval and Charrin, the constant current destroys the diphtheria toxin,[129] while the high-frequency current causes molecular changes in the toxin, but they were unable to make diphtheria antitoxin by this method. Bonome and Viola, using this method, claim to produce an antitoxin to streptococci.[130]

According to Ostromisslenski[131] all antitoxins are globulins. The specificity of an antitoxin is determined by the physical condition of its colloid particles and only the physical condition of an antitoxin distinguishes it from normal globulins. In some cases globulins may combine with toxins by means of heat alone. Thus if the toxin of certain staphylococci is heated with rabbit serum at 60, the globulin is converted into an antitoxin,[131] but usually this required catalytic agent. Diphtheria antitoxin is claimed to have been made from normal horse serum or globulin of eggs by means of a catalisk (NaCl)[131] but this work has not been corroborated and as yet the question of the chemical nature of this antitoxin remains unsettled. According to Berg and Kelser's work the evidence points to a nonprotein character for the tetanus antitoxin.[132]

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[127] Wassermann, A.: Ztschr. f. Hyg., **18:** 235, 1894; Brieger, L., and Ehrlich, P.: Ztschr. f. Hyg., **13:** 336, 1903.

[128] Brieger and Ehrlich.

[129] d'Arsonval and Charrin: Comp. rend. hebd. Soc. de biol., **3:** series 3, 96, 121, 153, 719, 1896.

[130] Bonome, A., and Viola, G.: Centralbl. f. Bakt., **19:** 849, 1896.

[131] Ostromisslenski, I. I.: J. Russ. Phys. Chem. Soc., **47:** 263, 301, 1915. From J. Chem. Soc., **107-108:** 751, 1915

[132] Berg, W. N., and Kelser, R. A.: J. Agric. Res., **13:** 471, 1918.

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